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PHYSIOLOGICAL REVIEWS

VOL. V OCTOBER, 1925 No. 4

THE FUNCTION OF THE PLASMA PROTEINS

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The blood in its various phases has been a subject of study and observation for many years. Its changes have long been a diagnostic sign in the practice of medicine (33). A large literature has grown up on the blood corpuscles, their origin and destruction, on fibrinogen and blood-clotting, and the quantitative variation and significance of the other constituents, protein, sugar, lipins, non-protein nitrogen and other substances. In spite of this mass of knowledge, with the exception of fibrinogen, very little is known with regard to the origin or function of the proteins. One reason for this state of affairs lies in the character of the substances under investigation and the methods at our disposal for studying the proteins as well as the difficulty in tracing them in a structure made up of closely related substances.

The study of the plasma proteins has proceeded in waves—with each new method of characterization or of analysis has come renewed activity to determine the significance of the proteins. The recent additions to our knowledge are numerous particularly as the result of the introduction of simple physical measurements such as viscosity, refraction, rate of sedimentation of the red blood corpuscles and of micro-methods for determining the plasma proteins. The older literature has been reviewed by Hammarsten (51) and more recently by Morawitz (99) and Berger (9), (13).

The evidence on the function of the plasma proteins is limited and uncertain. Method and the immediate significance of the results obtained play an important rôle in the study of the question and will continue to do so. We have felt justified, therefore, in examining the procedures now in vogue in an attempt to evaluate them for the purpose of understanding what has been done and of stimulating further enquiry. So much new material has accumulated that it has not been possible

nor has it seemed necessary to go back to the earlier work, from a bibliographical point of view, to develop the present state of our knowledge.

The standpoint from which this discussion is written is that the protein fractions separated by salting out are chemical entities or mixtures of such entities. This attitude is assumed because there seems to be more evidence in favor of it than there is for the belief that the fractions are the accidental products of the mode of separation or are separated simply on the basis of their colloidal behavior. The evidence from salting out is more in the direction of differences in solubility as at present understood than of differences in colloidal states.

The plasma proteins as chemical and physical entities. The question of the individuality of the proteins of the plasma is fundamentally one of the interpretation of the results which have accumulated by the classical methods of preparing the various protein fractions (salting out and acidification) as evidence of the separation of an apparently homogenous fluid into chemical entities or the differential precipitation of colloidal particles of various degrees of dispersion.

The early work on the proteins was based on the hypothesis that there were substances of different constitution in the blood. This was a natural assumption since the most characteristic protein of blood, fibrinogen, separated from the rest of the plasma in an insoluble form, fibrin, without the aid of special chemical manipulation. Fibrinogen had a definite property, that of coagulating. There was also a second protein, euglobulin, having properties in common with fibrinogen but without that of coagulating spontaneously, such as insolubility in water, solubility in dilute salt solutions (precipitation on dilution) and precipitable by the careful acidification of the plasma; particulaly after dilution.

Other protein fractions with more or less definite properties have been obtained from plasma through the use of the temperature of coagulation and salting out with salts of various concentrations. Thus we have, in addition to fibrinogen and euglobulin, pseudoglobulins, albumin, possibly more than one albumin (104), (47), and certain less recognized and somewhat problematical substances such as fibrinoglobulin and seromucoid. The distinction between euglobulin and pseudoglobulin was the solubility of the latter in water and the higher concentration of salt required for its precipitation. Thus euglobulin was precipitated by a saturated solution of sodium chloride, a one-half saturated solution of magnesium sulfate or a one-third saturated solution of ammonium sulfate, while pseudoglobulin was not precipitated by a saturated solution of sodium chloride and required a saturated solution of magnesium

sulfate and a one-half saturated solution of ammonium sulfate for its precipitation. Albumin, on the other hand, was soluble in water and was precipitated only by saturation with ammonium sulfate, or upon acidification of a saturated solution of magnesium sulfate, or the addition of another salt, such as sodium sulfate.

The work of the Hofmeister school extended the number of salts which could be used to fractionate protein mixtures and showed the relation between various salts when used to precipitate euglobulin from serum. The limits between which various proteins were precipitated were defined in terms of the percentage of a saturated solution of ammonium sulfate. The limits for the proteins vary slightly with different investigators; in general they are (percentage of a saturated solution of ammonium sulfate) (116): fibrinogen, 30 per cent; euglobulin, 30 to 36 per cent; pseudoglobulin I, 36 to 44 per cent; pseudoglobulin II, 44 to 50 per cent; albumin, 50 to 100 per cent.

A recent application of the salting-out procedure for the determination of plasma proteins (66), (72) has resulted in the expression of the limits of precipitation of the various fractions in terms of fractional volume-molar concentrations of different salts. After the first fraction, fibrinogen, has been precipitated the additional amount of salt required for a subsequent fraction is a constant for any salt. This increment of salt may be different for different salts. The quantities of protein precipitated from blood plasma agree very well with the results obtained for certain fractions by the older procedures, such as recalcification in the case of fibrinogen, dilution and acidification with carbon dioxide in the case of euglobulin, and half saturation with ammonium sulfate in the case of total globulin. A study of the solution and precipitation of the proteins of muscle (74) indicates a relation between the proteins of muscle and those of blood based at present entirely on the increment of salt required for solution or precipitation. We have then a continuous series of salt concentrations required for the precipitation of body proteins from those which precipitate the proteins of muscle, some of which require a lower concentration for precipitation than do the plasma proteins, down to the concentration which causes the complete precipitation of all the proteins of plasma or muscle. The concentrations are given in table 1 (74):

The presence of different albumin fractions is a subject which has received little attention. Certain observations indicate that there are different albumin fractions in blood plasma, as follows: different heats of coagulation and the variable distribution of albumins according

TABLE 1

Volume-molar concentrations of various salts required for the equal precipitation of the proteins of blood plasma (fractions I to IX) with the probable concentrations for muscle proteins (fractions A to D).

SALT	Increment					GLOBULIN FRACTIONS OF BLOOD*				ALBUMIN FRACTIONS OF BLOOD*				
		D	C	B	A	I	II	III	IV	V	VI	VII	VIII	IX (Total protein)
Na_2SO_4	0.25			0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00	2.25	2.50	2.75
$(NH_4)_2SO_4$; $ZnSO_4$	0.25	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00	2.25	2.50	2.75	3.00	3.25
$MgSO_4$	0.375		0.225	0.50	0.875	1.25	1.625	2.00	2.375	2.750				
Li_2SO_4	0.40			0.20	0.60	1.00	1.40	1.80	2.20	2.60				
$\frac{KH_2PO_4}{K_2HPO_4}$: $\frac{1}{2}$	0.30		0.225	0.525	0.825	1.125	1.425	1.725	2.025					
$\frac{NaH_2PO_4}{Na_2HPO_4}$. $\frac{1}{2}$	0.30			0.30	0.60	0.90	1.20	1.50	1.80					
NaCl	1.25			1.25	2.50	3.75	5.00							
LiCl	1.00	1.00	2.00	3.00	4.00	5.00	6.00	7.00	8.00					

* Determined at 37°C. for blood proteins. It is necessary to work at 34–37°C. when using sodium sulfate and for the higher concentrations of sodium phosphate.

to the species of animal, demonstrated by heat coagulation (47); the separation of fractions having definite limits of precipitation (104); the fraction specificity of albumin fractions (30); the variation in the albumin fractions under environmental conditions (75), and the well known fact that it is impossible to crystallize out all of the albumin of serum.

The regularity of the increment, common to all salts, raises the question of the significance of the protein fractions separated by salting-out. It is possible that we are not dealing with the precipitation of individual proteins but with a phenomenon of the periodic precipitation of protein which reflects the activity of the salt or to a periodic reaction between colloidal particles and the added electrolyte. Cohn (26) has recently discussed the solubility of proteins in salt solutions. For the particular observations under consideration the significance of the fractions rests upon *a*, the similarity of quantitative determinations of nitrogen precipitated at the concentrations used with those obtained by other procedures; *b*, the absence of euglobulin and pseudoglobulin I from the blood of new-born calves and the appearance of these fractions upon the ingestion of foods containing these fractions, such as colostrum or blood serum (68), (70); *c*, the absence of euglobulin from the blood of the new-born infant (91), (18), and *d*, variations in one fraction without marked variations in other fractions (70,) (75), (76). In addition to this evidence, which is largely of biological origin, there is a considerable amount of data as to differences in chemical structure and of immunological specificity of certain of the fractions (150).

The practice of using volume-molar solutions has, as yet, little more experimental justification than the use of saturated and fractional saturated solutions. It has the merit of expressing the concentration of salt in a more rigorous manner and of eliminating any possible implication that the solubility of the salt has any particular significance in the salting-out of proteins. Furthermore, it is not any more diffcult to prepare such solutions than to prepare saturated solutions. In a comparison of procedures and different concentrations of salts it is well to remember that any concentration of salt will give consistent analytical results when used in the same manner.

The removal of salts by electrodialysis is being used to separate the water-insoluble globulins from the water-soluble albumins. Ruppel (128) has thrown down euglobulin by electrodialysis of serum between membranes of different character to prevent changes of reaction of the dialysate; a negatively charged (vegetable) membrane on the cathode side and a positively charged (animal) membrane on the anode side.

Pseudoglobulin was then separated by salting-out or by the differential dialysis of the pseudoglobulin from the albumin. Electrodialysis according to this procedure has led Stern (143) to the conclusion that there are two parts to the euglobulin fraction, the CO_2 precipitable fraction, which he calls the labile globulin, and that portion which requires the complete removal of electrolyte for its precipitation. This observation may find its explanation in that of Pauli (106), (107) who found by careful and prolonged electrodialysis of serum to the conductivity of distilled water that all of the protein usually considered as globulin (precipitation with one-half saturated ammonium sulfate, pseudoglobulin plus euglobulin) can be thrown down. Pauli used parchment membranes and holds that membranes of two kinds are not necessary. The determination of the antitoxin content of protein precipitated by electrodialysis between parchment membranes (2) showed that the antitoxin content of the precipitate remained fairly constant for a time and then suddenly increased with an increased precipitation of protein. This increase in antitoxin content was associated largely with the separation of the fraction which has usually been classed as pseudoglobulin. All antitoxin was thrown out of solution with the completion of precipitation. The conclusion drawn from this work and that of Pauli is that the distinction between a water-soluble and a water-insoluble globulin no longer exists. The globulin (pseudoglobulin) considered as water-soluble in reality requires a small amount of salt to hold it in solution, an amount of salt which is not removed by ordinary dialysis.[1]

In considering the fractionation of the plasma proteins we must not lose sight of the fact that the various means employed to precipitate proteins are capable of throwing down other protein substances not ordinarily found in the blood or which are present in very small amounts. It is only because other proteins do not seem to appear unchanged in appreciable amounts that we are justified in accepting our present methods of analysis or preparation as giving relatively definite "fractions." Hemoglobin appears to be precipitated largely with either the globulins or albumins according to the species of animal.

The influence of the lipins on the precipitability of the plasma proteins is another field which has not been adequately studied. Lipins

[1] Serum of the new-born calf which contains neither euglobulin and essentially no pseudoglobulin I likewise gives a precipitate upon electrodialysis (76). In these experiments the precipitate did not appear until after the solution had become alkaline and then acid. It is possible that the changes in reaction may be a factor in the precipitation of pseudoglobulin by electrodialysis (155).

extracted from horse or ox serum precipitate the serum from which they have been extracted. Native, dialyzed, horse or ox serum, whether or not it contains lipins, is more readily precipitated as the lipin concentration increases (52). Soaps appear to form combinations, possibly loose, with the serum proteins and to give precipitates with serum proteins (77), (94). It is possible that euglobulin is a complex of lecithin and pseudoglobulin (23). There appears to be an inverse relation between the quantity of cholesterol which can be extracted from serum with ether and its euglobulin content (54); the phenomenon is affected also by the kinds and proportions of salts present.

The recent work of Sörensen (139) on the solubility of the globulins has raised a question as to the significance of the salting-out procedures. The assumption is made that euglobulin and pseudoglobulin—he does not consider more than the two globulins—occur in serum not as mixtures but as combinations of the two globulins. This is apparently a different assumption from that suggested by Hardy (59) that the fractions do not exist in the natural serum as entities but as a complex unit which is dissociated by salting-out. The combinations which Sörensen describes are equilibrium conditions between a euglobulin-pseudoglobulin complex in solution and the precipitated complex, if present, which may be formed or dissociated after the fractions have been separated from the blood serum. Important questions in this connection with regard to fractionation which will need to be answered are: What is the extent of the dissociation of euglobulin from pseudoglobulin in salting-out? and, What are the conditions for the maximum dissociation of these proteins, or of other globulin or albumin fractions, from each other? Sörensen's classification of euglobulin and pseudoglobulin as the proteins precipitated by a one-quarter saturated solution and by a one-half saturated solution of ammonium sulfate, respectively, (which we assume is tentative), or euglobulin as the protein precipitated by dilution with water, adheres to salting-out on the basis of classification and has not taken into account the work of Pauli referred to above. The solution of the problems opened up by Pauli and by Sörensen will result in a clarification of the situation as to the significance of the protein fractions and of their interrelations.

The chemical and immunological evidence of the individuality of protein fractions has been presented by Wells (150) and will not be further discussed here.

Methods employed for the estimation of changes in concentration of plasma proteins. Salting-out. After the choice of the kind of salt to be used has been

made, see above, the estimation of the various plasma proteins varies according to the method of determining the quantity of protein precipitated. The oldest method consisted in filtering the precipitated fractions on weighed filter papers, washing the precipitate with the proper concentration of salt solution and then coagulating the protein with hot water, washing the coagulated protein, drying and weighing to a constant weight (120), (42), (151). Instead of weighing the precipitate the nitrogen content of the washed coagulated protein may be determined by the Kjeldahl method (61). These procedures are particularly adapted to the estimation of protein when ammonium sulfate is used as the precipitant. Fibrin may be estimated by recalcification of oxalated or citrated blood, after which the fibrin is dehydrated with heat or with alcohol and ether (44), (71) and then weighed to a constant weight, ashed and the protein determined by difference (35), (135), or without ashing (44), (71); these procedures are particularly convenient when fibrinogen is the only plasma constituent to be determined.

Instead of estimating the protein precipitated by a salt it may be determined indirectly by coagulating the unprecipitated protein and weighing or by determining the nitrogen content of all, or an aliquot, of the filtrate (24), (67) see however (72), (61). When a non-nitrogen containing salt is used for the original precipitation, e.g., sodium sulfate, magnesium sulfate, a mixture of the mono- and di-basic phosphates, or lithium sulfate, the indirect method may be used for rather extensive and ready fractionation of small quantities of serum or plasma. The objections to such procedures are that they depend upon differences between determinations with an increased possibility of error. The adaptability and greater range of usefulness of such procedures justify their use when the limitations are appreciated.

Colorimetric procedures have been employed for the estimations of protein concentrations either in the original precipitate or in the filtrates. The colorimetric procedure of Wu (154) seems particularly promising.[2]

Various turbidimetric methods have been proposed most of which are adapted to clinical tests.

The value of any of the salting-out procedures depends in the first place upon the significance of the fractions obtained. At present most of our evidence as to protein fractions is related either directly or indirectly to separations by salting-out. Until independent procedures are devised for identification of the proteins, salting-out must remain as the point of departure for future work. The relative proportion of serum to salt solution is of great importance in the estimation of protein fractions as well as in their preparation (116), (151), (66), (10), (42). With the usual plasma or serum at low dilutions, 1:1 to 1:5, it is probable that more protein will be precipitated than at higher dilutions, 1:10 or above (151). The explanation of this condition is found, in part at least, in the observations of Spiro (140) and of Sörensen (138), (139) that salt is concentrated in the supernatant liquid above a precipitate, i.e., the precipitate contains less water and less salt for a given volume than the precipitating liquid above it. With colostrum rich in euglobulin the concentration of salt in the superna-

[2] Perlzweig (108) has found the procedure of Koch and McMeekin (84) to be satisfactory for filtrates from sodium sulfate precipitations in blood and in urine, including urines containing sugar.

tant liquid, at a dilution of 1:4, has been observed to be sufficient to precipitate the next fraction, in this case caseinogen (76). The globulin precipitate of serum at a dilution of 1:1 may ocupy $\frac{1}{4}$ to $\frac{1}{2}$ of the total volume (10).

The addition of acid to complete a precipitation of protein presents certain difficulties, too much or too little acid will dissolve or fail to precipitate it and thus lead to low results. A recent study in which the protein was precipitated by salting-out and the coagulation of the filtrate was used (120), (121) indicates that where care is taken with regard to the degree of acidity very good results may be obtained by coagulation in the presence of acid.

Unless care is taken in the collection of the blood and in the separation of the plasma or serum fictitious changes in the concentration of the protein may occur. The addition of oxalate through its effect on the distribution of water between the plasma and the cells will cause an increase in serum protein (109). Stasis, cold and other unusual conditions may cause a transitory increase in protein. Long contact of serum with the clot from which it has separated will give an apparent shift in the total protein. Such changes may be due to a shift in the water and salts between the blood corpuscles and the plasma, although these changes are relatively insignificant (40), (89). Such changes, such as CO_2 or oxygen loss, may be particularly evident in refractometric and viscometric determinations. Hemolysis will increase the apparent quantity of the albumin or the globulin fraction according to whether the hemoglobin is precipitated with the albumins or the globulins. There may be a slight change in the relative proportions of albumin and globulin in the direction of an increased albumin content without apparent hemolysis (76) a shift which has not been adequately studied.

Two methods have been used in the quantitative estimation of the different protein fractions: *a*, the precipitation of different portions of plasma each at a given concentration of salt, i.e., simultaneous precipitation, and *b*, the successive removal of the fractions from the same portion of plasma. In the first case all fractions precipitated by the particular concentration of salt are removed in the single precipitation. The results are obtained by difference and are open to the additional error of the inaccuracies of the associated determinations. For the extensive fractionation of small quantities of plasma the procedure is very valuable. The successive precipitation of the fractions offers many difficulties from a quantitative point of view in that allowances must be made for losses in volume by filtration and changes in salt concentration as the result of the separation of the precipitate referred to above. This procedure has recently given satisfactory results (4).

Estimations of plasma or serum proteins by physical methods. The refractometer has been used to determine the concentration of total protein and of the protein fractions of plasma and serum. The refractive index of one protein fraction does not differ markedly from that of another (10). This procedure offers the possibility of making rapid and rather accurate determinations of the quantity of protein present in a solution—with suitable corrections for the salt present. The results tend to be too high when compared with determination by the Kjeldahl procedure (10), (49), (54), (92); the greatest error occurs in blood plasma with a high globulin content (10).

Robertson ((122), see also (10), (54)) has developed the most comprehensive system of analysis around the refractometer which requires as little as one and

one-half cubic centimeter of serum for the determination of euglobulin, pseudoglobulin, albumin and non-protein nitrogen. This procedure carries with it all of the difficulties with regard to separation of the fractions which exist in the case of salting-out as well as those of the refractometric determinations. The separation of the globulins from the albumins is made by the addition to the serum of an equal volume of ammonium sulfate. At this low dilution there will be a tendency to cause a greater precipitation of protein than should occur because of the increase in the concentration of salt in the supernatant liquid. This error is in part compensated (it is probably over-compensated) by the correction for the ammonium sulfate used in precipitation, since the correction is based on the dilution of a corresponding amount of the original sulfate solution; such a dilution will probably have a lower refractive index than the supernatant solution from the precipitation of the globulins.[3]

The interferometer has been used in place of the refractometer for the estimation of the plasma proteins because of its greater accuracy. The results are not particularly promising in dilute solutions (46).

The relative proportions of albumin and globulin in blood serum have been estimated by a correlation of the refractive index and the viscosity ((119); critique and literature (10), (49)). This method is rapid and requires but small amounts of serum. It is based on the differences in the specific viscosities of solutions of globulin and albumin and the fact that the refractive indices of these two types of protein are approximately the same and are additive (see, however, (10), (46), (54)). From a chart (102), (119) based on studies of purified protein fractions the relative proportions of albumin and globulin can be obtained. A comparison of the refracto-viscometric procedure with Robertson's method and the salting-out method (10), (14), or by salting-out with magnesium sulfate (40), indicates that the method gives fairly concordant results for normal sera but that for pathological sera the values are generally too high. The procedure does not appear to be suitable for the absolute determination of the protein fractions; it may be of considerable clinical value because of its simplicity, particularly when used for comparative purposes, but even in such cases one must be careful in his interpretation. The results when properly evaluated may be significant (141), (10), (14), (49), (50) either of themselves or when contrasted with other procedures.

There is an extensive literature relating to the physical or physical chemical condition of the blood or blood plasma or serum and disease, particularly for diagnostic purposes. The viscosity of the serum is not related alone to the relative concentrations of albumin and globulin but is affected by other more or less undefined factors. The realization of this condition has been followed by attempts

[3] One, not very conclusive, estimation of these errors has been made (101). The change in the quantity and concentration of salt was made by washing the precipitated protein with one-half saturated ammonium sulfate and then determining the change in the concentration of the wash liquid. A marked change in concentration was not found. This evidence is not, however, sufficient since only a small amount of solution remains with the precipitate and, furthermore, the readjustment of the equilibrium with the new concentration of salt would be small.

to utilize viscosity measurements for the study of disease on an empirical basis and for the study of the colloidal state of the plasma (112). Thus we have the specific increase in viscosity (111) which is the relation of the observed viscosity to the viscosity of normal serum of the same protein content obtained from Naegeli's chart (102), (119), (123) which gives the relation between viscosity and the concentration of protein. A measure of the "colloidal condition" of serum has been proposed (50) which is expressed as the difference between, or the ratio between, the viscosity of the unknown serum and the normal viscosity (from Naegeli) both corrected for the viscosity of the dispersion medium. Various other phenomena have been in part correlated with changes in the concentration and relative proportions of the globulins and albumins which are in general empirical. Among these are, in particular, the suspension-stability, "Senkungsgeschwindigkeit," of plasma measured as the rate of settling of the red blood corpuscles (Fahreus (33), Berczeller, Wastl, Linzenmeyer, Starlinger, Westergren), the determination of the gold number (Reitstötter), and evaluations of the colloidal stability of the plasma or serum proteins, particularly for diagnostic purposes, such as are contained in the work of Schade, Weltmann, Klausner, Sachs and Öttinger, Frisch and Starlinger, Gerloczy, Daranyi, Starlinger, Mayer, Luger and Weiss and Oestborn.

Variations in the concentrations of the proteins of the plasma. The concentrations of the plasma proteins may vary with *a*, the species; *b*, age; *c*, sex; *d*, feeding; *e*, physiological activity; *f*, environment, and *g*, disease and the reactions to infection. Under ordinary conditions the composition of the plasma of an individual is relatively constant. Changes in the concentration of fibrinogen seem to occur most rapidly. There is an interrelation between the lymph and tissues and the blood plasma about which we know very little. The changes in endothelial permeability (110) under the influence of epinephrin and of shock indicate possibilities of at least a temporary modification in blood plasma concentration through such action.

The evaluation and correlation of the various observations to show absolute differences in the distribution of the plasma proteins is difficult because of the variety of procedures which have been used in their estimation with the accompanying variations in results. The general trend of changes which take place in abnormal conditions such as disease can be deduced from comparative results with almost any of the methods employed. To single out definite values as representative of particular states, and especially the normal composition of the blood plasma, is exceedingly uncertain. The majority of the investigations relate to the fractionation of serum into the globulins and the estimation of the albumin-globulin quotient. The largest number of analyses on different animals and in disease have been made with the refracto-viscometric procedure of Robertson.

TABLE 2

The distribution of the protein fractions in the plasma or serum in various species of animals. Results expressed as grams of protein in 100 cc.†

	TOTAL PROTEIN	FIBRINOGEN*	SERUM PROTEIN*	EUGLOBULIN	PSEUDOGLOBULIN		TOTAL GLOBULIN	ALBUMIN				TOTAL ALBUMIN	ALBUMIN / GLOBULIN
					I	II		V	VI	VII	VIII-IX		
Chicken, 2 (74)			3.60	0.61	0.54	0.63	1.78	0.46	0.72	0.38	0.26	1.82	50.6:49.9
Brahma bulls, 8 (55)			7.66	1.68	2.78		4.46					3.20	41.8:58.2
Cow, 9 (74)	7.43	0.72	6.71	0.78	1.85	0.87	3.50	0.35	0.76	1.78	0.31	3.21	47.8:52.2
Dog, 3 (17)	6.06	0.58	5.48	0.46	1.20	0.66	2.32	0.57	0.52	0.64	1.42	3.16	57.7:42.3
Goat, (4)			7.03	1.88	1.38		3.26					3.77	53.7:46.3
Goat, 4 (116)	6.91	0.60	6.31	0.46	1.97		2.28					4.02	64.0:36.0
Guinea pig 40 (74)	4.80	0.33	4.37	0.32	0.53	0.49	1.34	0.45	1.98	0.40	0.20	3.03	69.3:30.7
Hog (4)			6.30	2.40	1.87		3.27					2.03	38.0:62.0
Horse, 2 (74)	6.10	0.34	5.76	0.46	1.37	0.65	2.49	0.72	1.87	0.63	0.05	3.27	56.6:43.4
Horse, 24 (115)			7.23	0.87	3.28		4.17					3.01	41.6:58.4
Human, normal woman, 13 (110)	7.49	0.30	7.19				2.73					4.45	62.0:38.0
Maternal 12, (110)	7.05	0.43	6.62				2.66					3.96	59.8:40.2
Fetal, 12 (110)	6.12	0.27	5.85				2.21					3.64	62.0:38.0
Rabbit, 1 (74)			5.55	0.35	0.22	0.89	1.45	0.95	0.20	2.57	0.37	4.10	74.9:26.1
Rabbit (4)			6.05	0.61	0.97		1.58					4.47	74.9:26.1
Sheep, 3 (74)	5.74	0.36	5.38	0.72	0.84	0.75	2.31	0.43	0.64	1.49	0.51	3.07	57.1:42.9

* The percentage of albumin and globulin is based on the total serum protein and does not include fibrinogen.

† The figure immediately after the name of the species of animal represents the number of determinations averaged to obtain the result given; the next number refers to the bibliography.

The distribution of the protein fractions in the plasma or serum in various species of animals is contained in table 2.[4]

These data are presented with the realization that except in cases where a large number of individuals have been examined over a considerable period of time and under conditions in which the animals are known to be relatively free from recent infection that they may represent accidental observations. The relative proportions of total globulins and albumins are in general consistently different in certain animals (see also 136). In man, sheep, goat, rabbit, dog, guinea pig and rat the albumins predominate over the globulins whereas in the horse, hog and cow the relative proportions of these two classes of proteins are nearly equal or the globulins tend to be in excess of the albumins. The limited data available indicate that there are distinct differences in the amounts of albumin fractions VI and VII (table 1) in the plasma of different species. Albumin fraction VI is greater than fraction VII in the horse, chicken and guinea pig while the opposite is found in man and the cow, sheep and rabbit. Bodansky (17) has found fraction VIII to be present in the greatest amount in the dog. The available data on man up to 1916 (125) have been collected by Rowe.

There are definite differences in the distribution of the plasma proteins at different ages. The young animal has as a rule a lower total protein concentration and a lower proportion of globulin than the adult (1), (18), (31), (54), (68), (70), (75), (88), (91), (118), (145), (146), (147). Plass (115) on the other hand finds the percentage of total globulins and albumins in fetal and maternal blood, mother and child, to be approximately the same, in spite of the lower total protein content of the fetal blood. The data show the interesting fact that the concentration of albumin is approximately the same in both maternal and fetal

[4] In presenting data on the composition of "normal" animals only data obtained by salting-out in which the plasma or serum was diluted have been included. The available data of Howe (76) have been used, with the realization of the small numbers of animals examined in some cases, because of the more extensive fractionation of the plasma and to bring out differences in the albumin fractions. The values for *total globulin* and *albumin* given agree well with other data (4). The extensive data of Robertson and his associates on mammals (78), (153) and domestic birds (19), (144) have not been included because, on comparison with the older data and the more recent data, with the exception of the rabbit and the horse, the concentrations of the albumin fractions are invariably high (see also 136). We ascribe this difference to a consistent error as indicated above. We do not intend to imply in not using these data that the procedure is valueless. Changes obtained when the method is used on successive samples of blood from the same animal are valuable on a comparative basis.

blood. The concentration of total protein in the premature infant is less than that of an infant at term and does not attain the normal value until about the age of three months (147). The ingestion of colostrum or serum causes a definite increase in euglobulin (91), (18) in the human infant and of euglobulin and pseudoglobulin I in the calf (68), (70). The data of Duzár and Rysznyak (31) indicate a high globulin content in the plasma of infants during the first two months, these authors have apparently not taken into consideration the effect of colostrum on the composition of the blood of the young infant. In the calf the absorbed globulin disappears gradually until at from 4–6 weeks of age the composition of the plasma is the same whether or not colostrum has been absorbed directly soon after birth. These changes are illustrated in the accompanying chart.

Various species of animals apparently react with different intensity to infection as indicated by the extent of changes in the plasma proteins and total protein; the horse and rabbit appear to react with greater changes than the cow which may show relatively slight changes under the influence of infection (4), (76).

Sex differences in the concentration of protein in the serum (48) (rats) and in the distribution of the fractions are not particularly evident (human) (1), (39), (guinea pig) (76), in fact they have not been adequately studied.

During pregnancy there is apparently an increase in total serum protein which is chiefly in the globulin fraction of women (49), (see however (87), (100), (114)) and particularly in the fibrinogen fraction (39), (44), (25). In the cow, on the other hand, pregnancy does not appear to have any marked effect on the concentration of the plasma proteins

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Fig. 1. Curves showing the variations in various protein fractions of blood with increasing age. Calf 898 did not receive colostrum, calf 899 received colostrum relatively low in globulins, and calf 669 received colostrum rich in globulins. The crosses (×) indicate results obtained on calves which did not receive colostrum; some of these animals were injected with cow serum, some were injected and fed cow serum, and some were fed cow serum. The results on these animals agree essentially with those obtained on animals which had not received colostrum or which would have received milk containing very small amounts of globulins. The plus signs (+) indicate results on animals which had received colostrum soon after birth. Average data for calves 6 and 12 months of age, non-pregnant heifers 17 to 22 months old, and pregnant heifers 30 months old are plotted as indicated in the legend. The data are presented in terms of grams of nitrogen in 100 cc. of blood plasma or serum. (Journal of Biological Chemistry, 1922, lii, 486.)

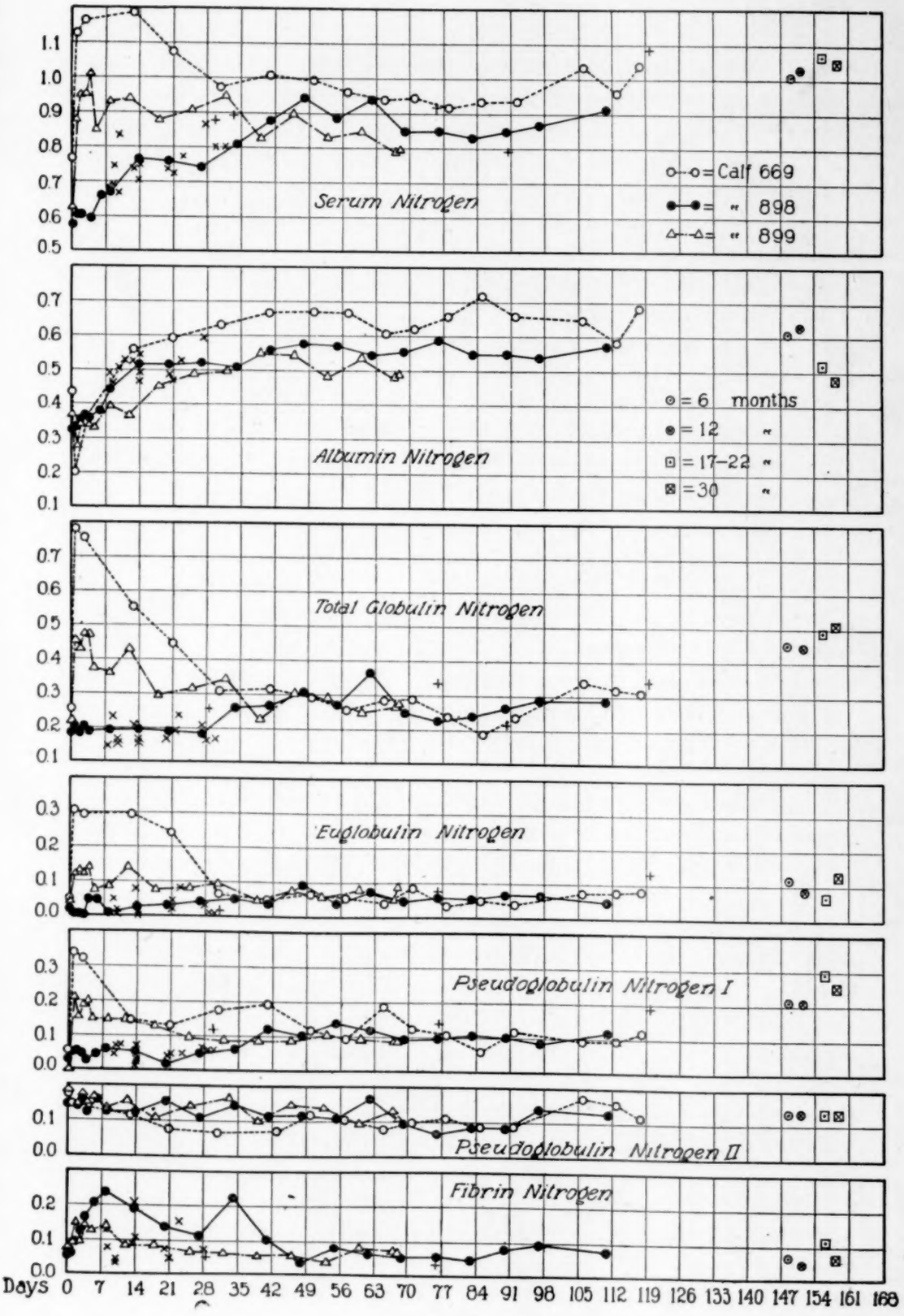
Serum Nitrogen
o-o = Calf 669
●-● = " 898
△-△ = " 899
Albumin Nitrogen
⊙ = 6 months
● = 12 "
⊡ = 17–22 "
⊠ = 30 "
Total Globulin Nitrogen
Euglobulin Nitrogen
Pseudoglobulin Nitrogen I
Pseudoglobulin Nitrogen II
Fibrin Nitrogen
Days 0 7 14 21 28 35 42 49 56 63 70 77 84 91 98 105 112 119 126 133 140 147 154 161 168

Fig. 1

(70) except in cases of infection or abortion (75). Fluctuations in the total protein content of serum in the course of pregnancy in women have been described (114) which are ascribed to changes in the water content of the blood. A decrease in the total protein was found (114) which was due almost entirely to a drop in the albumin fraction, while the globulin fraction just about maintained its level and the fibrin rose throughout the course of pregnancy (115).

Food has little effect upon the composition of the blood (1), (56). There are transitory changes in the total proteins of the serum following the ingestion of water. Such changes persist for but a short time and soon return to the usual value, in a half hour to an hour. Deprivation or sudden loss of water from the body is followed by a concentration of the plasma proteins (95), (147) which is soon restored in the normal individual upon the ingestion of water. Various observations have been made upon the distribution of the plasma proteins in fasting which indicated an increase in the total protein; it appears, however, that the reported changes are probably the result of individual variaton (55) or of loss of water (146), (147) and not of the deprivation of food. Deprivation of both food and water results in a concentration of the total protein of the serum (146), (147) which seems to be chiefly the result of the loss of water. Changes in the rate of metabolism under the influence of metabolic accelerants (thyroid extract) or depressants (quinine or sodium cacodylate) do not have an effect on the ratio of albumin to globulin (56).

There are changes in the concentration and distribution of the serum proteins which cannot be ascribed to the effect of food, or of disease, e.g., seasonal or environmental variations. The albumin content of the sera of the ox has been found to be highest in summer with accompanying changes in the globulin content (54), (57). These observations need to be verified by an extended study of a group of individuals or animals. Fluctuations in albumin fraction VII have been observed (75) which may be related to temperature changes but which do not have an adequate explanation at present. Changes in body temperature have little effect upon the albumin-globulin quotient (56). There are, however, fluctuations in the concentration of the blood in the course of heat regulation (5). Light may have an effect since by irradiation with ultraviolet and Röntgen rays there is a shift in the refractive index of the serum (85), which appears to be more than a simple change in the water balance of the blood. Studies with salting-out procedures have

not shown any effect of light on the distribution of the protein fractions (108).

The most marked variations in the concentration of the plasma proteins accompany the reaction of the body to infection or disease. Changes in the total protein and of the varous protein fractions and particularly fibrinogen and in the ratio of albumin to globulin or the protein quotient have been the subject of study for many years. Isolated determinations of the plasma proteins have not been particularly significant in diagnosis or progress of disease or of functional condition of the individual. Consecutive determinations of these substances on the same individual seem to offer greater possibilities in studying the course of disease, and have yielded the most significant results. At present the value of the determination of changes in the plasma proteins lies particularly in the field of research.

Evidences of a shift in the water-balance of the body in disease have been based on variations in the total protein concentration of plasma or serum. The dilution or concentration of the plasma could well take place by the increase or decrease in the water content. The determination of the total protein concentration of the plasma or serum is not, however, necessarily indicative of hydremia or anhydremia. Sufficient evidence has accumulated to demonstrate a variation in one protein fraction without a marked change in another fraction. Thus there are the rapid increases and independent variations in the fibrinogen content of the plasma (137), (70), the successive changes in the protein fractions upon immunization (9), the independent variations in the blood of the calf (70), the distribution of the protein fractions in nephritis (92), and the differential rate of restoration of the serum proteins after plasmapharesis (81), (82), (83). With such evidence one is not justified in assuming a simple change in the water content of the blood from changes in the total protein without additional proof.

The scope of this paper does not permit us to go into a detailed discussion of the variation of the concentration of total protein and the plasma protein in disease. The literature on human blood serum has been reviewed by Rowe (125), (126) and briefly by Myers (101), and the general field has been summarized and discussed in its relation to tissue changes by Berger (9), (13).

Origin of the plasma proteins. The site and mode of the origin of the plasma proteins are practically unknown. The inter-relation between the blood, lymph and body cells makes it difficult to single out any par-

ticular part of the body as responsible for the formation of the blood constituents. That the protein is of cellular origin is certain; the question is whether or not the fractions arise as identities, with perhaps independent origin, or have a common origin and are inter-related and finally the immediate source of the proteins. The "life cycle" of the proteins is equally obscure. An examination of the development of the chick embryo (130) indicates that in this case the blood vessels arise from cells called the angioblasts which differentiate from mesoderm and produce endothelium, blood plasma and red blood corpuscles, i.e., that the first blood plasma results from the destruction of cells and is different in origin from the tissue-fluid. Doctor Sabin (131) does not believe that this mode of origin can be ascribed in the adult to the endothelium of the blood vessels but that it is possible that the endothelial liver tissue might perhaps continue to function in this way. She has other suggestive evidence (131) in the periodic disintegration of neutrophilic leucocytes. These cells might be a source of a part at least of the blood proteins. Foster (39) and McLester (96), however, in studying fibrin did not find a parallelism between fibrin and the leucocyte curve in all cases of lobar pneumonia.

There are certain facts with regard to the plasma proteins which point to some mechanism which determines either the production or utilization (or both of these states) of the plasma proteins, such as: *a*, the relative uniformity of the total protein concentration and of the distribution of the protein fractions under similar conditions; *b*, the concentrations and distribution of the protein fractions vary with the species of animal but are relatively constant for a given animal and species; *c*, there are distinct differences with regard to age and the mode or rate of increase of the fractions; *d*, environmental factors may produce fluctuations in one fraction without marked changes in another; *e*, under the stimulus of infection or immunization decided changes may be produced in the total protein concentration and the fractions which persist or may be effaced; *f*, the gradual disappearance of a marked increase in the concentration of a protein fraction.

The effect of the ingestion of food and of water has not been found to produce more than a transitory change in the quantity or distribution of the proteins of the plasma. Hemorrhage is followed by a gradual restoration of the fractions—most rapid with fibrinogen, followed by the globulins and finally the albumins (81), (82), (83), (137).

Fibrinogen shows the most transitory and varied fluctuations in concentration of any of the proteins and to such an extent that it is believed

(137), (70), (110) to have an independent origin and fate. This belief is not, however, shared by all who have studied the question as will be evident in the subsequent discussion of the origin of the protein fractions. The most recent work (35), (36), (37), (38), (39), (96), (135) on fibrinogen indicates the site of formation of this protein to be almost exclusively the liver, with reserve supplies in the body, probably in the intestinal tract. The only condition which causes or is associated with low fibrin values is acute extensive liver injury. Tissue injury which ordinarily causes a stimulation to fibrin production is unable to obliterate the fall in fibrinogen concentration which accompanies liver injury. These conclusions agree with much of the older work (99). An increased production of fibrinogen takes place upon tissue injury and inflammation (38) produced by either sterile or infected abscess formation, toxic proteoses, Röntgen rays, acute infections, or small doses of liver poisons. It is possible that dietary factors may be concerned in fluctuations in fibrin observed upon feeding. The rapidity with which fibrinogen is restored after hemorrhage in contrast to the slower regeneration of the other plasma proteins (137) is evidence in favor of the separate origin of fibrinogen. Mathews (93) is opposed to the idea of the liver as the chief source of fibrinogen. He inclines to the hypothesis that fibrinogen is formed either in the blood-forming organs, the bone marrow (see 99) or by the disintegration of the leucocytes and possibly the red cells.

The evidence with regard to the site and origin of the serum proteins may best be discussed under two headings: *a*, that which relates to the serum proteins as an aggregation or modification of the albumin fractions into the more complex globulins, or the converse as derivatives of fibrinogen (a more complex protein, a highly hydrated protein or a protein aggregate of a low degree of dispersion, according to the conception of the structure of a protein fraction); and *b*, that which relates to the serum protein fraction as produced individually by the cells.

a. Evidence relating to an inter-relation between the protein fractions. The hypothesis of Moll (98) that globulins are formed from albumins has more experimental evidence against it than in its favor (34). Both chemical and immunological evidence on the individuality of the protein fractions is opposed to such an idea. The original observation is based upon changes in solubility; a lower concentration of ammonium sulfate was required for the precipitation of albumin solutions after they had been made alkaline and then heated to 56° to 60°C. for an hour than before such treatment. Various other investigations have confirmed

this observation at lower temperatures (128), (129) and even without the addition of alkali (46). In the present state of our knowledge with regard to changes in the physical properties of protein preparations under the influence of heat (156), acidity, alkalinity (155) and purification we are not justified in assuming on the basis of changes in solubility (changes which often result in the assumption of true colloidal behavior) that there has been a change from one protein fraction to another, where protein fraction is used to imply the fraction originally separated from blood serum or plasma. In other words, the physical characteristics of protein preparations change so readily that the burden of proof rests upon the investigator to show that a change in solubility is not the result of "denaturization."

The tentative observation (23) that the protein fraction of euglobulin and pseudoglobulin is identical and that euglobulin is a complex of pseudoglobulin and a phosphorus compound, probably lecithin, is a more logical interrelationship. There is certain evidence to support this hypothesis in the similarity of the composition of the protein portion of the two globulins with regard to the distribution of amino acids (58) and in the ultraviolet absorption spectra (90).

An attractive but unsubstantiated hypothesis as to the origin and interrelation of the plasma proteins is that of Herzfeld and Klinger (62), (63), (64). The assumption is made that the protein fractions separated by salting out or otherwise do not have a chemical individuality but are an interrelated series of colloidal particles of different degrees of dispersion, which can be transposed one into the other. The evolution begins with the lowest dispersed particles, fibrinogen, and extends to the albumins and non-coagulable substances. The protein particles are stabilized by the absorption of lower products of disintegration, such as amino acids and polypeptides. The smaller and more soluble particles carry the greatest amount of absorbed material. The origin of the plasma protein is explained as the disintegration of the cell proteins into debris which, in turn, give rise to fibrinogen, etc. The processes of synthesis take place in the cells where the specific protein is built up upon a characteristic pattern. The whole conception is highly speculative. The experimental evidence to support this hypothesis is limited. In the case of the change from fibrinogen to globulin the evidence is decidedly open to question. Kapsenberg (80) has recently accepted this hypothesis in part, in so far as there may be a difference in the "globulins" and "albumins" in the sense of Herzfeld and Klinger. They

consider, however, that the nucleus of the globulin and albumin are different.

Starlinger (142) sees in the thyroid gland a site for the degradation of the proteins of low degree of dispersion on the basis of a higher concentration of fibrinogen and rate of sedimentation of the red corpuscles in blood going to the thyroid gland than coming from it, and an increase in fibrinogen in hypo-function and after thyroidectomy. Such changes are assumed to be in accordance with Herzfeld and Klinger's conception of the activity of a secreting gland (62). The experimental data are not very convincing and should be extended.

The globin of the red blood cells has recently been advanced by Reymann (120), (121) as the source of at least a part of the blood proteins, particularly the fibrinogen and globulin increase which occurs in the process of immunization. The observation is made that during immunization to diphtheria toxin the formation of antitoxin is accompanied by a decrease in hemoglobulin and of the number of erythocytes. This decrease is followed, first by an increase in fibrinogen and this by an increase in globulin; there is an accompanying decrease in albumin. The reciprocal change in albumin and globulin is believed to be due, in part at least, to the fact that there is a limit to the total concentration of protein in the plasma. On this hypothesis an undue increase in globulin necessitates a decrease in the albumin fractions. The fact that the hemoglobin of the horse is precipitated by one-half saturated ammonium sulfate is offered as supporting evidence to the hypothesis. The hypothesis of Herzfield and Klinger is tentatively accepted as the basis for the interrelation between the various protein fractions.

There are certain objections to this idea of Reymann's particularly in so far as the similarity between the precipitation limits of pseudoglobin and hemoglobin are concerned, since hemoglobin is apparently precipitated at different concentrations of salt for different species of animals. This subject has not been carefully studied. The use which has been made of the Herzfeld and Klinger hypothesis, while only tentative, is as questionable or valid as that hypothesis may ultimately prove to be. On the other hand there is the possibility, which is admitted but not enlarged upon, that the increase in the "globulin" as the result of the immunization of the horse may be an increase in globin as such; this might be so since the precipitation by salts does not distinguish between two proteins with the same precipitation limits and horse hemoglobin is apparently precipitated with the pseudoglobulin fraction.

Should the Reymann hypothesis be substantiated the evidence on the balance between the formation and destruction of the red blood corpuscles (127) will be important as to the constancy of the composition of the plasma. In this case the hematophage apparently of endothelial origin may play an important part in the transformation of globin to the plasma proteins which may be correlated with the disintegration of the neutrophilic leucocytes (131).

b. Evidence relating to a more or less independent origin of the plasma proteins. A progressive change in the concentration of fibrinogen, globulin and albumin (refracto-viscometric method) in rabbits (9), following the injection of protein and in disease (15) has been described. The sequence is an increase in fibrinogen (assumed from the literature), later an increase in globulin followed by a drop in the concentration of globulins, and finally an increase in albumin. This shifting of the distribution of proteins may extend over a long period of time, as much as four months.

With an increase in globulin (hyperglobulinemia) or albumin (hyperalbuminemia) there is an increase in total protein (hyperproteinemia). Such changes are true alterations in the concentration of the proteins and not the result of concentration due to loss of water. The variations in the concentration of the plasma proteins in nephritis (92) also indicate fluctuations in total protein and the protein fractions which are not the result of the simple increase or decrease in the water content of the blood. A genetic difference between the various fractions is deduced from the fact that *a*, the increases are not simultaneous; *b*, that the transition is not gradual but sudden, and *c*, the length of time over which the transition may take place. These changes have led Berger to the conclusion that changes of the protein content of the blood are regulated by the same laws as the other blood constituents. An increase is looked upon as the result of an increased release of protein from the cells, and that an increase in globulin in the blood may be the result of an increased formation of globulin in the cell. Recent studies of the proteins of muscle, liver and kidneys (12), (16) are in progress in an attempt, among other things, to find a relation between the proteins of the tissues and the body fluids; so far no positive evidence has been obtained. A greater proportion of globulin was found in the liver and kidney, but there was a predominance of albumin over globulin in extracts of the muscles in the rabbit. Changes in the relative proportion of albumin and globulin in the blood plasma and organ extracts following protein injection tended to be in opposite directions and seemed to indicate that there was not a

direct relation between the tissues and the blood. Gutzeit (45) has studied the question of the relation of the tissue proteins to the plasma proteins by analysis of the fluids obtained by perfusion of the animals (rabbits) with 0.9 per cent NaCl solution and analyses of the perfusion liquid and of the muscles, liver and kidneys for total protein, albumin and globulin. He found in the final perfusion liquid and in the tissues a greater concentration of globulin than of albumin, an increase which was greater than existed in blood serum. The conclusion is reached that the replacement of serum albumin by plasmapharesis is not accomplished by a direct passage of unchanged fluids from the tissues to the blood stream, but must arise in some other undetermined manner. These studies of Berger and Gutzeit represent serious attempts to determine the relation of tissue protein to the blood proteins. The methods of estimating the proteins of the tissues and the significance of the fractions are so uncertain that the deductions are open to question, as Berger has clearly intimated (see also 74). Linder, Lindsgaard and Van Slyke (92) have offered an hypothesis which is essentially similar to that of Berger. They suggest that an increase of globulin may be a phase of metabolic derangement, that the formation of plasma globulins is the primitive form of protein production to which the body returns in disease.

A difference in the rate of formation of globulin and albumin, the former more rapidly than the latter, is indicated by the results of Whipple and his co-workers in their studies of protein regeneration by blood plasma depletion or plasmapharesis. The reconstruction of these plasma proteins appears to require as great a time interval as it does to repair and replace liver cells following extensive liver injury. The site of production of the serum proteins seems to be the liver, although other tissues may be concerned, but the mechanism of formation appears to be different from that of fibrinogen. Regeneration takes place during fasting; it is stimulated, however, by the ingestion of food (82). The relative difficulty with which the serum proteins are regenerated when the normal level has been lowered suggests that the normal wear and tear of the proteins must be slight (9). The sequence of plasma protein regeneration found by Whipple is similar to the variations in plasma protein fractions observed by Berger (9), i.e., fibrinogen, globulin, albumin.

Gottschalk and Nonnenbruch (43) have presented evidence which indicates that the blood proteins are formed in the tissues in general and that the liver is not exclusively concerned in the formation of these proteins. They base these conclusions on studies in which frogs show a

passage of plasma protein into the blood stream after the extirpation of the liver and an artificial lowering of the protein content of the blood. Furthermore, the content of amino acids in the portal and hepatic veins was approximately the same during absorption of amino acids from the intestinal tract.

The apparently independent changes in the distribution of the protein fractions of blood plasma in the young calf either in the normal changes or those following inoculation suggest the independent origin of the plasma proteins. Thus, there is a gradual increase in albumin from birth (there may be a slight drop very soon after birth) which occurs without regard to the changes in the globulins; a large increase in fibrinogen is not followed by a subsequent increase in the other globulin fractions (see fig. 1) and independent fluctuations occur in the albumin fractions which do not seem to be related to changes in the globulin fractions.

A correlation of the evidence presented in support of the different views with regard to the origin of the plasma proteins is not readily apparent because of the differences in species, age and, possibly, environmental conditions of the animals worked upon and to a certain extent in the technic employed. The horse gives greater fluctuations in its protein fractions than the cow (4); and apparently its hemoglobin has different precipitation limits; conditions which exist in the young animals appear to be different from those of the adult animal (75). It does not, however, seem logical to assume that the fundamental processes should be very different between species and at different ages. Upon such an assumption it is difficult to harmonize an interrelation of protein fractions suggested by Herzfeld and Klinger and by Reymann (which, he informs me, is only tentative and emphasizes the fact that his work is with the pathological animal) with the apparently independent origin of the proteins observed by Whipple and his co-workers, by Berger, and by Howe. The relatively independent origin of fibrinogen seems to be the most certain factor in all of these uncertainties. An hypothesis which has no direct experimental support is that the greater concentration of globulin, particularly in the adult as compared with the young animal is the result of a readjustment of the protein equilibrium following the usual infections which occur in all animals (75).

We are left with no idea as to how the constancy of the proteins is maintained nor how the changes from the condition characteristic of the young animals to that of the adult are to be accounted for. The mechanism by which the concentration of protein is maintained is one

of equal importance with the origin of the proteins. We can do little else than call attention to some of the phenomena. The young animal begins life with a low concentration of total protein, with the absence of one or more of the "fractions," and possibly a different distribution of the albumin fractions. In the course of its development an increased amount of protein and a different distribution of the fractions is attained. In cases of infection there is usually an increase in fibrinogen and often an increase in one or more of the fractions. The fibrinogen appears rather quickly to return to a normal after the infection is over, but the distribution and amount of the other fractions may persist for a longer period of time. There is usually a tendency to return toward the normal after the immediate stimulation of the infection has disappeared. It would appear, however, that the original conditions are not always reëstablished; it was this fact which led us to offer the hypothesis in the preceding paragraph. The length of time required for the removal of the excess protein thrown into the system is almost an unknown quantity. Most long-time studies of blood changes have involved repeated injection without complete recovery, in the present sense, before a second injection or after the last one. What occurs in the calf which has ingested colostrum or blood serum (70), (73), (75) may not represent the condition in the adult although the fragmentary results following immunization (9) indicate that there may be a similarity. In the calf, following the rapid accumulation of globulin by direct absorption, there is a gradual decrease in concentration which extends over 28 to 40 days, which would indicate that the absorbed protein was being treated as an extraneous protein and that its rate of removal was limited in some way. Changes in the immunization of the rabbit (9) may extend over 20 to 40 days.

In case it is found that there is a relation between the disintegration of the red blood corpuscles (127) and of the leucocytes (131) the evidence of a rather uniform, even though cyclic in the case of the leucocyte, rate of destruction of the cells will be of importance.

The function of the plasma proteins. Fibrinogen, of all of the plasma proteins, has an obvious function in its relation to the stoppage of hemorrhage, both internally and externally, through its change to the insoluble fibrin; coagulation. Attention has been called (37), (38) to the relation between the lack or failure of the blood to clot and the appearance of bleeding in man and animals, e.g., about the teeth, in the stomach and intestines, rupture of capillaries through pressure and the black and blue areas which follow trivial bruises. Fibrin deposits are conspicu-

ous and probably continuous in acute inflammations (pneumonia, peritonitis, abscesses) and it is probable that they occur in actual necrosis (hyalin liver, cell necrosis and infarcts). There appears to be a constant daily consumption of fibrinogen by the body, while unusual demands for fibrinogen, such as hemorrhage, inflammation and injury call forth an increased production.

Fibrinogen and euglobulin, particularly the former are concerned in the suspension stability of the formed elements of the blood (33) for a high fibrinogen content is associated with a more rapid rate of sedimentation. The tendency of the red blood cells to rouleaux formations is increased most by fibrinogen. The effect of the globulin has been ascribed to a change in the colloidal state of the blood; an increased viscosity; albumins have a much less effect than the globulins.

The oldest conception of the function of the plasma proteins was that they were the form in which the nitrogenous nourishment was transported from the intestines to the cells. This hypothesis involves direct absorption or the synthesis of protein from digestion products in the intestinal wall or perhaps in the liver. The direct absorption of the food proteins, even of homologous proteins, does not appear to occur extensively except in the very young and no convincing proof of immediate synthesis has been presented. The demonstration of an increase in amino acids in the blood and tissues following the ingestion of proteins has led to the belief that the nitrogenous nutrition of the tissues is accomplished without extensive resynthesis of the absorbed digestion products into blood proteins. The slowness with which the *serum* proteins are regenerated after plasmapharesis is believed (81) to be further proof that they are not actually concerned as transition products of protein metabolism between food proteins and body tissue proteins.

Evidence that the serum is an indirect source of food for cells is found in the work of Carell (20). Serum is not itself a culture media for the growth of fibroblasts and epithelial tissue *in vitro*. Certain constituents of embryonic juice or aqueous extracts of leucocytes are required for the proliferation of the tissue. The serum becomes an indirect source of food trephones for these tissues through the action of the leucocytes. These cells may be considered as unicellular glands which transfer constituents of the serum into trephones and set them free in the plasma and lymph as a food for fixed cells.

The "cellular activity" of the body is influenced by the presence or absence of the plasma proteins. Reductions in the concentration of the plasma proteins of the dog by plasmapharesis (152), in which the red

blood cells are suspended in Locke's solution and introduced simultaneously with the removal of blood, if carried sufficiently far, results in "shock" and death. If, however, the cells introduced be suspended in normal dog serum or in dog serum which has been dialyzed against Locke's solution no "shock" results. A moderate reduction of the plasma proteins does not necessarily result in shock. The presence of injured cells of the kidney, pancreas or intestine does not seriously modify the course of the reaction. Injury of the liver cells (chloroform of phosphorus poisoning), on the other hand does modify the reaction of the animal such that fatal shock accompanies even a moderate plasma depletion. These experiments are offered by Whipple and his coworkers as evidence that the plasma proteins have a stabilizing protective effect upon the body cells, and also that the liver is particularly concerned in the "shock" reaction.

The use of acacia in blood transfusion (6) in man is other evidence of the need of a stabilizing factor in the blood. The pharmacological effects of blood serum in perfusion experiments are apparently the result of changes which take place in serum after it has been removed from the body (27), (53).

Schade (132), (133) in discussing the distribution of water between the blood, connective tissues and cells has emphasized the stabilizing action of the blood on the tissues in general. He presents evidence to show that the shift of water between the red blood corpuscles and the plasma proteins under the influence of changes in reaction are antagonistic and thus tend to maintain the "onkotic" —swelling capacity—constant. Krogh (86) has suggested a relation between the height of an animal and the degree of dispersion of the colloids of the blood, i.e., their effect on osmotic pressure.

There are other evidences of the action of the plasma proteins in stabilizing the activities of the body. Variations in the rate at which the red bood corpuscles settle out upon standing is related to the concentration of the globulin and particularly fibrinogen (33). Such a phenomenon occurs in the capillaries when the blood flow is sluggish; different individuals give different effects, e.g., it occurs more rapidly in a pregnant woman than in a non-pregnant one. The plasma proteins have a rôle in the acid-base equilibrium of the blood (97), (148) which is of minor importance when compared with the electrolytes and hemoglobin.

The problem of the changes in the degree of hydration of the serum or plasma proteins (colloids) and the effect of such changes on the water

balance and excretion of urine is at present a subject of discussion. The assumption is made (32) that the amount of water held by the plasma proteins or of tissues is affected by changes in hydrion concentration, salts and organic substances. An increase in viscosity, swelling, results in the retention of water while a decrease in viscosity is followed by diuresis, e.g., the effect of caffein. Changes in water held by the blood may be the result of an increased concentration of total protein as well as increased hydration of the proteins. The relation between the amount of water obtained by ultrafiltration (7) and edema and diuresis would seem to support this thesis. The rôle of the plasma protein as an important factor in the water regulation has, however, been questioned (103), (54) on the basis of evidence which failed to show changes in viscosity (hydration) and refraction (103), concentration of protein and that the degree of alkalinity (greater than pH 9) required to cause dehydration is not compatible with life. Studies of the swelling pressure ("onkotiscker druck") of the blood plasma have led Schade (132), (133), (134) to the conclusion that renal diuresis is the result of a decreased attraction of the body colloids for water, in which it is not the colloids of the tissues which are particularly concerned but the colloids of the blood plasma. A decreased swelling pressure of the blood plasma as the result of kidney insufficienty permits the passage of water into the connective tissue; edema. A constant relation was not found between swelling pressure, refractive index and viscosity of blood plasma.

The function of the plasma proteins in their relation to the antibodies, developed in response to immunization, is not clear—the simplest statement of the facts is that the globulins act as carriers of the antibodies. Extensive investigations of the development of immunity have shown that there is, at least, a casual relationship between the globulins of the plasma and the immune substance (9), (121), (150), (80). The antibodies appear to be definitely associated with one or another of the groups of globulins. They are precipitated with certain fractions by salting-out, acidification or electrodialysis even though another fraction is removed first. There is not a change in the association of the antibodies with certain protein fractions during the passage of protein through the gastro-intestinal tract in the direct absorption of protein into the blood stream of young animals (105). An interrelation between an increase in the concentration of globulins in the blood and the degree of immunization has repeatedly been sought without success. It is certain that there is usually an increase in the globulin fractions of the

blood as a reaction to the injection of toxin or of antigen. The reactivity of animals differs—the horse seems to be particularly reactive, while the cow may be sluggish in showing an increase in protein upon immunization. On the other hand, the extent of the globulin increase is not a measure of the degree of immunization. An immunity may develop without an increase in globulin, and vice versa. The site of formation of agglutinins seems to be the liver (79), the organ which the evidence suggests is also the site of formation of the plasma proteins.

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The letters used in connection with the bibliography have the following significance:

A, Salting out, dilution 1:10 or greater.
B, Salting out, dilution less than 1:10.
R, Robertson, Refractometric procedure (117).
R-V, Refracto-viscometric method of Reiss (114) or Naegli-Rohrer (119).
I, Immunological evidence.
Wu, Wu's colorimetric procedure (148).

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BASAL METABOLISM OF CHILDREN

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The first recorded study of the basal metabolism of infancy and childhood is that of Andral and Gavarret in 1843 (1). Many investigations have been carried on since then but only those that have some particular bearing on basal metabolism from the modern point of view will be considered in the text. Many of these early works form the foundation stones upon which have been laid the successive steps of our knowledge of basal metabolism but they are not all comparable with modern data because all the qualifications of basal metabolism were not fulfilled. A synopsis of the early literature on basal metabolism is given in Benedict and Talbot's publications (12), (13), (16) and the references included in the bibliography appended herewith.

The present chapter in the history of basal metabolism may be said to have opened in 1898 and 1899 with the work of Rubner and Heubner (102), (103). These investigators used a modified Pettenkofer-Voit apparatus especially constructed to study the metabolism of infants. Its construction was such that only periods of several hours could be measured, and most of the data published was not obtained under basal conditions.

Schlossmann (116) in 1908, using the Zuntz and Oppenheimer modification of the Regnault-Reiset apparatus, observed a great difference in the heat production of an infant when absolutely quiet periods were compared with active periods. Relatively short periods of three to four hours were studied but this apparatus was so constructed that the experiments could not be interrupted. The apparatus consisted of a hermetically sealed glass chamber through which air was circulated from an outside circuit. This chamber was immersed in a tank of water in such a manner that the infant was always visible.

The next studies of importance were made by Howland (60) in 1913. He measured the heat production of infants in the Atwater-Rosa-Benedict apparatus of Professor Lusk at Cornell University Medical School.

This apparatus measured the heat elimination as well as the heat production. Howland's work was of special importance because it showed that heat production calculated from the gas exchange was always within 3 per cent and usually within 2 per cent of the heat elimination as directly measured, in subjects with a constant body temperature. His data are given in table 1.

Accepting Howland's findings that the estimation of the metabolism by the indirect method gives results very close to those obtained by the

TABLE 1

Heat production of infants directly and indirectly measured

SUBJECT	FOOD	CALORIES PER SQUARE METER PER DAY		
		Measured (direct)	Calculated (indirect)	Difference
				per cent
Child 1	Ordinary	1,046 1,113 1,196	1,084 1,174 1,164	2
Child 1	Nutrose added	1,218	1,179	3
Child 1	Nutrose added	1,204 1,235 1,181	1,180 1,212 1,250	Less than 1
Child 1	Fasting	1,106 1,226 1,301	1,177 1,156 1,243	Less than 1
Child 3	Ordinary	858 913	793 933	2
Child 3	Ordinary	825	840	2

From Howland: Direct calorimetry of infants,—Trans. 15th Internat. Congress on Hygiene and Demography, Washington, 1913, ii, 438.

direct measurements of heat, Benedict devised a respiratory chamber for infants and a cot chamber for older children in which a large number of observations have been made by him and Talbot (12), (13), (16) on infants and children.

The infant respiratory chamber is so constructed that short periods may be studied, and it may also be used for twenty-four hour determinations.

In 1915 Murlin (81) also working in Lusk's laboratory devised a metabolism incubator which he used attached to the Benedict universal absorption table. This apparatus is so constructed that it can measure accurately periods as short as one-half hour in duration.

The general principles of metabolism have been extensively reviewed in the books of Lusk (69), Grafe (53) and DuBois (41) and the monographs of Boothby (32), McCann (76), Murlin (86) and Edgard Zunz (169). The following paper, therefore, will not attempt to review all the work in the field of basal metabolism, but will consider only that relating especially to infancy and childhood.

A decade ago basal metabolism studies of infants and children were carried on in only a very few clinics. Today an increasing number of laboratories are operating or installing respiratory chambers for the purpose of studying the metabolism of childhood. All the important work on early childhood has been carried on in respiratory chambers; that on later childhood mostly with the portable apparatus.

The portable apparatus, when properly controlled, may be a useful and accurate means of measuring the basal metabolism of older children and adults but is of doubtful value in young children. Metabolism studies at the Massachusetts General Hospital on children have led to the conclusion that measurements obtained by the portable apparatus are less likely to be basal the younger the child, chances being decidedly against basal periods in a child under eight years of age. For this reason only studies obtained in the respiratory chamber in early childhood, and selected and well controlled data obtained by the portable apparatus in later childhood will be included in this review.

The term basal metabolism is commonly used by all workers in the field. There is still some confusion in the way it is used due to the fact that standard conditions differ in some details in different clinics. Usually the accepted definition in childhood is the same as that used in adults. This statement must be qualified, however, because most of the observations on infants and children up to two years of age are with food in the stomach, while in older children they are post-absorptive. This distinction should always be borne in mind in reading the literature. All investigators have found it necessary to feed infants of less than two years before obtaining their metabolism. Benedict and Talbot (16) carefully state that "the basal metabolism of children under two years of age can thus be compared with that of older children only on the distinct understanding that the values for the basal metabolism for the younger children are higher than they normally would be owing to the

influence of food." Inspection of the standard curves shows that the curves are distinctly elevated before two years of age because of this factor. If due allowance is made for the increased heat production due to food the curves (excepting the first three to five days of life) up to the second birthday might be reduced ten to twelve per cent to be comparable with those obtained in older children without food.

A second feature which may complicate the metabolism data in early childhood is the greater frequency of sleep. It is possible to sleep comfortably in the cot chamber, and almost impossible to do so when a nose clip and mouth piece are used with the portable apparatus. It is quite possible that the low metabolism found in deep sleep may counteract to some extent the increase in the metabolism due to food. The effect of sleep on the metabolism will be discussed later.

Influence of food on the metabolism. Attempts have been made to determine the influence of food on the metabolism of infants by various investigators, more especially Howland (60), Murlin and Hoobler (82) and Benedict and Talbot (16).

Howland (60) caused an increase of 10 per cent in the metabolism of a three-month-old infant by adding 15 grams of nutrose (containing 14.25 per cent nitrogen) and an increase of 26 per cent in a seven-month-old infant by the addition of 30 grams of nutrose.

Murlin and Hoobler (82) estimated the "specific dynamic action" of mixed food in various infants to be between 10 and 12 per cent, while Benedict and Talbot (16) thought that the variation was between wider limits, 8 to 15 per cent. In the new-born, Murlin (88) concluded that food had a very slight "specific dynamic action" during the first eight days of life. Lactose and dextrose, he found, increased the metabolism about 12 per cent.

Talbot and Gamble (136) found that when the protein in the formula of a five-month-old infant was increased from 60 to 109 grams the metabolism increased 8 per cent.

Talbot[1] found that in cases of congenital stenosis of the pylorus, the post-operative metabolism with food was about 12 per cent higher than the pre-operative metabolism without food.

Talbot and Moriarty (148) found a slightly increased heat production in infants fed on dry milk powder. The deviation, however, was not sufficiently great to be of any striking significance.

The new-born infant is an example of presumable physiological fasting

[1] Unpublished data.

and subsequent feeding. If food is not previously given, the metabolism obtained on the second day of life should be comparable with that determined on the sixth day after a satisfying meal of human milk. The increase in the metabolism due to the "stimulating effect" of food, in new-born infants, is approximately 14 per cent, while in later infancy and childhood it varies within wider limits of from 8 to 15 per cent.[2] The increase in the metabolism, due to the stimulating action of food, is presumably influenced, as in the adult, by the amount and quality of the food taken. Although it is difficult to tell when the influence of the ingested food ceases, it is generally assumed that the "post-absorptive" state commences about ten to twelve hours after an ordinary meal.

Influence of sleep on the basal metabolism. Studies have been made on the basal metabolism of children during sleep, which is more common in young infants than in older children and the question arises as to how much does sleep affect the metabolism. This must be held in abeyance as yet because of the difficulties in obtaining sufficiently controlled data which are strictly comparable. Benedict's Girl Scout series suggests that there is a probable decrease in the metabolism during sleep but Benedict (23) was unable to find any convincing evidence that sleep affected the metabolism of girls between the ages of eight and twelve years. This phase of the subject must be studied in more detail before it is possible to say whether sleep lowers the basal metabolism, and, if so, how much.

The relation of growth and development to basal metabolism. Growth and development are factors which influence the basal metabolism and probably are responsible for differences met with in interpreting results. It is during the period from birth to puberty that the greatest changes in growth take place, the greatest gain being made during the first months of life and again at puberty. In adult life the body has reached its maximum development, the metabolism is standardized to the final physical characteristics of the individual, and is sustained during health at a fairly constant level until old age, when the intensity of life commences to diminish. The adult, therefore, is standardized for his type, while the child is continually changing and developing. Herein lies the explanation of many of the differences found during childhood and of the greater difficulties met with in interpreting the results.

The changes due to growth and development are practically met with from birth. During the first days of life an adjustment has to be made

[2] Deduced from data in Benedict and Talbot, Carnegie Inst. Wash., Publication. 233.

to the changed surroundings. Muscular tone and vigor are acquired and body heat is maintained if necessary by shivering, except in the case of weaklings whose body temperature, unless protected by clothes, quickly tends to approximate that of the surrounding air.

With advancing age the body increases in both height and weight. During this time the body surface also increases but at the same time becomes proportionately smaller in relation to the height and weight.

There is also increasing muscular development and activity. Instead of being confined to bed the child commences to move about and his type of life changes. With the increase in activity comes a change in shape and appearance; the body, with increasing age, assumes proportions which more nearly approximate those of the adult. Changes take place in the amount of active tissues, muscles and organs, as compared with inactive tissues, fat, bone and water; the former increasing and the latter decreasing. These changes all modify the metabolism findings.

As puberty is approached sexual characteristics develop and the influence of sex differentiation on the metabolism is apparent. The male becomes taller and more muscular with less fat, whereas the female is apt to add considerable deposits of fat to the body. At this time also various glands of internal secretion develop new activities or modify old ones, all of which tend to exert an influence on the metabolism to a greater or less degree.

It is obvious, therefore, that changes during childhood due to growth make this period one of constant change and it is necessary to take many factors into consideration in the interpretation of results. Benedict (23) suggests that Pirquet's pelidisi be adopted as a method of estimating mathematically the physical fitness of the child, and finds it valuable in establishing a straight line relationship. Up to date this procedure seems to be more valuable than $\frac{\text{WT.}}{\text{HT.}}$ relationship. It is found to be very useful method of comparison in the physiological study of the normal child, but should be always supplemented by clinical estimations of physical fitness in children who deviate from the normal or are pathological. It is not surprising that workers, in the adult field, who are in the habit of comparing results with narrow and perhaps inflexible standards, do not always look with sympathy upon the difficulties and complications met with in interpreting the results of metabolism studies during childhood.

I. Basal metabolism of normal children. *Heat production per*

square meter of body surface. Estimation of the heat production per square meter of body surface depends upon an accurate measure of the surface area of the body. Very few actual measurements have been made of the surface area of infants or children. Such formulas as have been given for estimating surface area represent mathematical rather than definitely established facts. It is probable, however, that they estimate very closely the true surface area of the normal child. No formula, however, which has not been controlled by enough actual measurements of the surface of normal children of different ages, height, weights and sexes is free from criticism.

TABLE 2

Estimated body-surfaces for body-weights from 3 to 40 kilograms

BODY WEIGHT (WITHOUT CLOTHING)	SURFACE* ESTIMATED FOR BOYS	SURFACE* ESTIMATED FOR GIRLS	BODY WEIGHT (WITHOUT CLOTHING)	SURFACE* ESTIMATED FOR BOYS	SURFACE* ESTIMATED FOR GIRLS	BODY WEIGHT (WITHOUT CLOTHING)	SURFACE* ESTIMATED FOR BOYS	SURFACE* ESTIMATED FOR GIRLS
kilos	*sq. m.*	*sq. m.*	*kilos*	*sq. m.*	*sq. m.*	*kilos*	*sq. m.*	*sq. m.*
3	0.208	0.210	16	0.711	0.686	29	1.086	1.048
4	0.252	0.255	17	0.740	0.714	30	1.110	1.072
5	0.292	0.295	18	0.769	0.742	31	1.134	1.095
6	0.330	0.333	19	0.797	0.769	32	1.159	1.119
7	0.388	0.388	20	0.828	0.796	33	1.183	1.142
8	0.424	0.424	21	0.853	0.845	34	1.207	1.164
9	0.459	0.459	22	0.879	0.872	35	1.230	1.188
10	0.492	0.492	23	0.906	0.898	36	1.254	1.210
11	0.524	0.534	24	0.932	0.924	37	1.277	1.233
12	0.556	0.566	25	0.958	0.949	38	1.300	1.255
13	0.586	0.597	26	1.009	0.974	39	1.323	1.277
14	0.616	0.627	27	1.035	0.999	40	1.345	1.298
15	0.645	0.657	28	1.060	1.024			

* Surfaces estimated, using formula $K \sqrt[3]{w^2}$†

† See Benedict and Talbot: Publication 302, Carnegie Inst., p. 62, et seq.

The DuBois linear formula (38) is based upon measurements from different parts of the body and therefore deserves more consideration than other formulas based only upon height and weight.

The Benedict and Talbot modification of the Lissauer formula (16), adapted to the DuBois linear formula, may be used in place of the DuBois linear formula in normal children where actual measurements are not practical. Estimated body surface measurements for body weights from 3 to 40 kilograms from Benedict and Talbot's modification of Lissauer formula are given in table 2.

These results are very close to those obtained from the DuBois linear formula and seem to be sufficiently accurate for use with normal children; the abnormal child, on the other hand, should always be measured according to the DuBois linear formula. It should always be borne in mind that the smaller the child the greater the chance of error in measurements. For example, it was found much more difficult to obtain consecutive readings that check in the premature infant than in the child old enough to coöperate. These factors must be kept in mind in studying the basal metabolism.

The basal metabolism per square meter of body surface for boys and girls as compared with adults (16) is presented in the curves in chart 1.

The heat production per square meter of body surface increases from birth to about eighteen months of age, or 12 kilos in weight, after which it gradually falls throughout childhood. In adults, on the other hand, there is little or no change in subjects of the same sex.

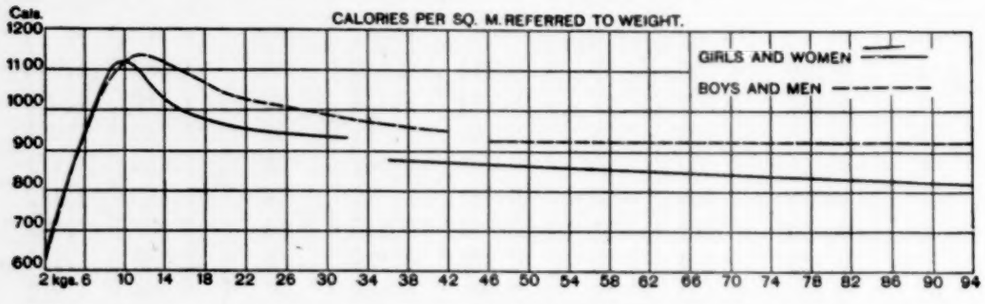

Chart 1

Although it is the general conception that the relation of the surface area of the metabolism is accidental, the standards of metabolism per square meter of body surface are considered valuable as standards of comparison because they are widely known and understood. They are, however, of less value in children since they are affected by deviations from the normal height and weight to a greater degree than in adults. A fat normal child will usually have a metabolism below the average and a thin normal above the average. The great majority of normal children, however, fall within 10 per cent of the average. It will be shown later that marked undernutrition, especially during infancy, may result in a metabolism which is seemingly elevated but which is actually lower than that seen in health as shown by the total heat production per twenty-four hours. The metabolism per square meter of body surface in the final analysis depends in part upon the weight of the child when any formula, except the linear formula, is used to esti-

mate body surface, and consequently the relative proportion of active and inactive tissue affects the final results. Since there is a greater proportional difference in weight and height in the child than in the adult the differences found in the metabolism of children and especially infants are more obvious.

A general discussion of the relation of surface area to heat production is to be found in chapter VIII of DuBois' excellent book (41). No explanation is given for the very low heat production found in premature infants, and new-born infants in whom the greater proportion of surface to the weight would lead one to expect a greater heat production. This unexpected relationship holds true up to the weight of 12 kilos in boys and 10 kilos in girls, or about one year of age, after which time the picture is reversed. In older children increasing weight is accompanied by a relatively decreasing body surface and heat production per unit of body surface. DuBois (41), however, says that "it is doubtful if any physiologists have seriously held the belief that the surface area was the immediate cause of the metabolism."

Carl Voit held that the mass of the cells and their power to oxidize materials determined the height of the metabolism and Benedict and Talbot (12) concluded in 1914 from their work on a large series of infants that the heat production is due to the amount of active protoplasmic tissue in the body. This opinion was not shaken by further studies of normal infants and children (13), (16) and has received confirmation from the work of other investigators.

Present opinion about the relation of body surface to adult metabolism is summed up by Boothby (32) as follows,—"Doubt concerning the relationship of the heat production and the surface area arose largely from a discussion of the applicability of Newton's law of cooling, and whether or not this could control the rate of heat production. The conception of control has been abandoned, however, by nearly all adherents of the applicability of the surface area law."

The metabolism per kilo of body weight. Body weight has been found to exert a profound influence on the metabolism. Heavy children have a low metabolism per kilogram of body weight, while thin children of the same age have a higher metabolism per kilo. This is illustrated in the three cases from the Benedict and Talbot series shown in table 3.

This illustration shows that the infants with the greater body weight, and presumably more subcutaneous fat, had the lower heat production per kilo of body weight. These figures seems to show that the metabolism per kilo of body weight is markedly affected by the composi-

tion of the body. Since fat may be looked on as an inert substance which plays little or no part in determining the amount of heat evolved in the healthy resting body, any additional weight added to the body in the form of fat will act as a diluent of the calories.

It seems fair to conclude that the composition of the body and especially the amount of active protoplasmic tissue, presumably protein tissue, is the principal determining factor of the height of the basal metabolism. As a result the total metabolism of infants of the same age, height and weight fall very close together. Fat, water and bony tissues are presumably inert substances and when the metabolism per kilo of two infants of the same heights but different weights are compared, the fatter infant is found to have a lower metabolism. The same holds true to a lesser degree when such infants are compared per unit of body surface (see table 3).

TABLE 3

*The influence of weight on the metabolism per kilo and per square meter of body surface of infants of the same age, sex and height**

BENEDICT AND TALBOT NUMBER	AGE	WEIGHT	HEIGHT	BODY SURFACE, DUBOIS LINEAR FORMULA	HEAT COMPUTED PER 24 HOURS		
					Total	Per kilo	Per sq. m.
	mos.	*kilos*	*cm.*				
129	3	7.07	62.0	0.380	311	44	828
130	3	6.02	63.0?	0.341	305	51	888
133	3½	5.81	62.5	0.327	329	57	1006

* From Benedict and Talbot, Carnegie Inst. Wash., Publication 302.

Metabolism per kilo referred to age. Chart 2 illustrates the trend of the calories per kilo as affected by age. Only the chart for boys is given as the curve for girls is similar though somewhat lower.

The calories per kilo increase during the first twelve or eighteen months of life, after which there is a gradual fall. An analysis of this chart showed that the cases falling below the lower line, which is 10 per cent below the average middle line, are fatter than the average child and those falling above the upper 10 per cent limit are thinner than the average child. During the first year the cases show a very much wider distribution. The sudden fall in the curve after the second year is presumably due to the fact that up to this time the metabolism, as noted earlier, was obtained with food in the stomach. After the second year the results are post-absorptive.

This statement, however, does not apply to children who are ap-

proaching puberty and have commenced to assume the adult development. They may then be short and fat or short and muscular and have different muscular development. The types of development must be taken into consideration when the proportion of inactive tissue in relation to the active tissue is estimated clinically. Unfortunately there is no accurate measure of the amount of muscle and body fat during life. Girls on maturing presumably have much more body fat than they had before the breasts and hips developed. The metabolism per kilo

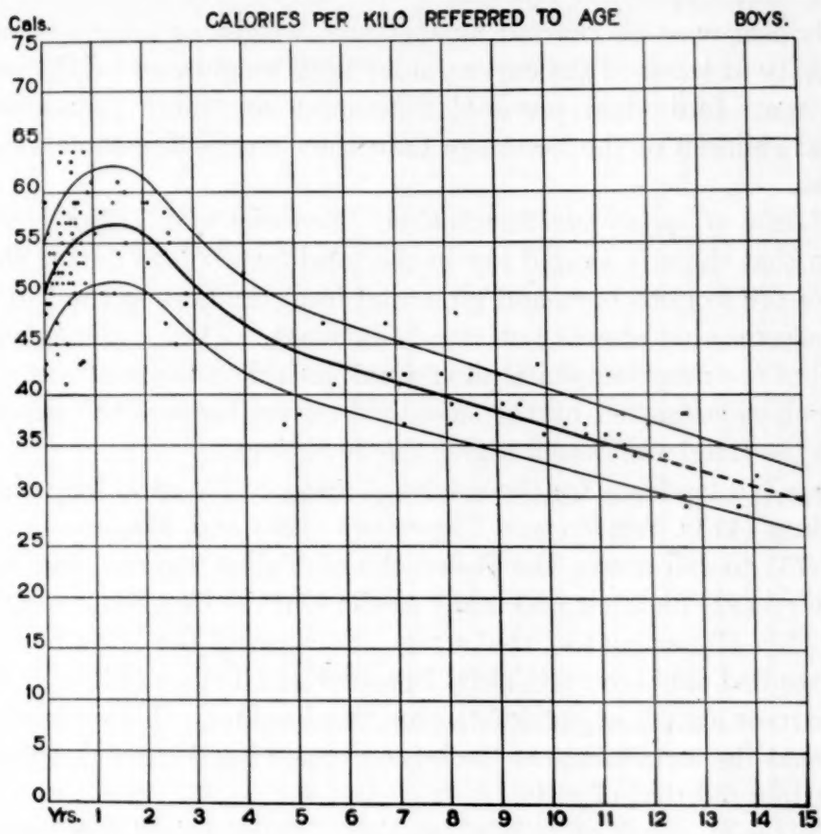

Chart 2

during childhood may be said to give an estimation of the relation of the inactive to the active tissues.

Benedict (23) says that the metabolism of girls from 12 to 20 years of age "may at present be best predicted from the curve based upon a series of experiments with Girl Scouts, showing the heat production per kilogram of body weight per twenty-four hours referred to age." This, however, does not in his opinion apply to younger and older females.

and the conclusion was reached with full appreciation of the influence of the relative proportions of active and inactive body tissues.

The difference of 30 per cent in the heat production of the infants in table 3 in which there is a difference of 21 per cent in the weight is quite different from DuBois's findings in adults (41) in whom there was only a difference of 22 per cent in the metabolism per kilo and nearly 100 per cent difference in the weight. This illustrates the greater differences in the metabolism of infants and children as compared to adults.

The total metabolism referred to age, weight and height. The total metabolism may be charted against age, weight or height showing a similarity of trend in the curves, since both weight and height increase with age. Individual physical differences are more pronounced in normal children of the same age than they are for the same height or weight.

Influence of age on total metabolism. Benedict and Talbot (16) have shown that there is a rapid rise in the total metabolism during the first year of life in both boys and girls, and from the first to the thirteenth year a somewhat slower but steady increase. They comment on the fact that too much emphasis should not be laid on the constancy of the metabolism suggested by the smoothed curves because the curve only shows the trend and does not give the deviations.

There is a tendency for the results of many of the early investigators, Scharling (113), Sondén and Tigerstedt (129) and Magnus-Levy and Falk (73) to fall above the Benedict and Talbot curve. Murlin and Hoobler's (82) findings, and many of the DuBois Boy Scout series (40) fall within 10 per cent of the curve. Because of the large number of cases studied (244 boys and girls) Benedict and Talbot (16) believe that their curves closely approximate true basal values. They found, however, that the metabolism of the boys grouped itself more closely to the curve than did that of girls.

Influence of weight on total metabolism. Since weight increases with age there is consequently a rise in the basal heat production with increasing body weight. Benedict and Talbot (16) found that boys deviated less from the smoothed curve than girls. They suggest that the metabolism for boys over 10 kilos may be predicted with an average deviation of ± 6.3 per cent by means of the total calories for the weight; children up to 10 kilos have a deviation of ± 8.7 per cent. For girls the deviation is somewhat greater. The prediction table of the total heat production of children of different weights is given in table 4.

Influence of height on total metabolism. The relationship between

total heat production and height seems to be closer for girls than any of the other methods of comparison. A possible explanation may be the difference in physical development and the amount of fat on girls of the same weight. Some are fat and short, while others are muscular, thin and tall. There is a similar but less marked difference in boys. Boys, however, tend to lead more uniform physical lives and consequently have a greater uniformity of physical development. The total

TABLE 4

*Basal heat production of boys and girls per 24 hours predicted from body-weight**

BODY WEIGHT (WITHOUT CLOTHING)	PREDICTED HEAT		BODY WEIGHT (WITHOUT CLOTHING)	PREDICTED HEAT		BODY WEIGHT (WITHOUT CLOTHING)	PREDICTED HEAT		BODY WEIGHT (WITHOUT CLOTHING)	PREDICTED HEAT	
	Boys	Girls		Boys	Girls		Boys	Girls		Boys	Girls
kilos	*cals.*	*cals.*	*kilos*	*cals.*	*cals.*	*kilos*	*cals.*	*cals.*	*kilos*	*cals.*	*cals.*
2.5	115	110	11.5	607	595	20.5	873	818	29.5	1103	1032
3.0	150	150	12.0	625	610	21.0	885	830	30.0	1115	1045
3.5	180	185	12.5	643	625	21.5	898	842	30.5	1127	1058
4.0	210	220	13.0	660	640	22.0	910	855	31.0	1140	1070
4.5	240	253	13.5	678	652	22.5	925	867	31.5	1150	1080
5.0	270	285	14.0	695	665	23.0	940	880	32.0	1160	1090
5.5	300	318	14.5	710	678	23.5	953	890	32.5	1170	
6.0	330	350	15.0	725	690	24.0	965	900	33.0	1180	
6.5	360	377	15.5	740	700	24.5	978	915	33.5	1190	
7.0	390	405	16.0	755	710	25.0	990	930	34.0	1200	
7.5	418	432	16.5	768	722	25.5	1005	940	34.5	1210	
8.0	445	460	17.0	780	735	26.0	1020	950	35.0	1220	
8.5	470	480	17.5	793	747	26.5	1033	962	35.5	1230	
9.0	495	500	18.0	805	760	27.0	1045	975	36.0	1240	
9.5	520	520	18.5	818	770	27.5	1058	987	36.5	1248	
10.0	545	540	19.0	830	780	28.0	1070	1000	37.0	1255	
10.5	568	560	19.5	845	793	28.5	1080	1010	37.5	1265	
11.0	590	580	20.0	860	805	29.0	1090	1020	38.0	1275	

* From Benedict and Talbot: Carnegie Inst. Wash., Publication 302, p. 206.

metabolism for height of boys, like that of girls, shows very little divergence from the smoothed curve. It seems probable, therefore, that the metabolism compared to height may prove the most satisfactory method of comparing the metabolism of both sexes. In order to make this available for use the predicted total metabolism for different heights of boys and girls is given in tables 5 and 6 obtained from the Benedict and Talbot data.

Influence of sex on the metabolism. The early investigators in their

work on the influence of sex on the metabolism unfortuntely disregarded the weight factor.

The work of Andral and Gavarret (1) on the influence of sex on the metabolism has been admirably presented by Sondén and Tigerstedt (129) in a discussion of their own researches. While the results of these

TABLE 5

*Basal 24-hour heat production of boys from the first week after birth to 12 years of age, predicted from height**

HEIGHT	PREDICTED HEAT	HEIGHT	PREDICTED HEAT	HEIGHT	PREDICTED HEAT	HEIGHT	PREDICTED HEAT
cm.	*cals.*	*cm.*	*cals.*	*cm.*	*cals.*	*cm.*	*cals.*
51	160	76	535	101	785	126	1000
52	170	77	555	102	790	127	1015
53	185	78	565	103	795	128	1025
54	198	79	577	104	800	129	1035
55	210	80	590	105	805	130	1045
56	225	81	600	106	810	131	1060
57	238	82	612	107	815	132	1072
58	260	83	622	108	820	133	1083
59	280	84	635	109	825	134	1095
60	300	85	645	110	830	135	1105
61	310	86	660	111	840	136	1115
62	315	87	670	112	850	137	1130
63	330	88	685	113	855	138	1140
64	358	89	695	114	865	139	1152
65	384	90	705	115	875	140	1165
66	390	91	715	116	885	141	1175
67	400	92	725	117	895	142	1187
68	420	93	730	118	910	143	1200
69	435	94	740	119	920	144	1210
70	450	95	745	120	935	145	1220
71	465	96	755	121	945	146	1232
72	480	97	760	122	955	147	1242
73	495	98	765	123	965	148	1255
74	510	99	770	124	980	149	1268
75	525	100	775	125	990	150	1280

* Talbot, F. B.: Not previously published.

investigators may not be considered basal, since modern conditions for basal measurements were not obtained, all their subjects were fairly quiet. Andral and Gavarret maintained that with boys the carbon dioxide exhaled increased suddenly to a large amount at the time of puberty, while with girls, on the contrary, the carbon dioxide excretion suddenly

ceased to increase at this period and remained nearly stationary until the menopause.

Benedict and Talbot in their studies (16) conclude that it is almost impossible to assert the presence of a pronounced sexual differentiation. From a consideration of metabolism curves on the different bases for men and boys and girls and women, it is evident that the metabolism

TABLE 6

Basal 24-hour heat production of girls from the first week after birth to 12 years of age, predicted from height (23)

HEIGHT	PREDICTED HEAT	HEIGHT	PREDICTED HEAT	HEIGHT	PREDICTED HEAT	HEIGHT	PREDICTED HEAT
cm.	*cals.*	*cm.*	*cals.*	*cm.*	*cals.*	*cm.*	*cals.*
48	122	71	483	94	630	117	837
49	136	72	500	95	637	118	847
50	150	73	516	96	644	119	857
51	165	74	530	97	651	120	866
52	178	75	543	98	659	121	875
53	194	76	557	99	667	122	885
54	208	77	567	100	675	123	894
55	222	78	575	101	685	124	904
56	236	79	583	102	693	125	915
57	250	80	586	103	700	126	925
58	268	81	591	104	711	127	935
59	283	82	595	105	720	128	945
60	300	83	598	106	730	129	956
61	318	84	602	107	740	130	965
62	332	85	605	108	749	131	975
63	350	86	607	109	759	132	985
64	367	87	610	110	769	133	995
65	384	88	612	111	778	134	1005
66	401	89	615	112	788	135	1016
67	418	90	617	113	797	136	1026
68	435	91	620	114	807	137	1037
69	452	92	623	115	817	138	1047
70	468	93	626	116	828		

of boys and men for practically the entire period of life is perceptibly and consistently higher than that for girls and women.

A comparison of the heat production of children of the same weight shows that there is little or no sexual difference between children of 11 kilograms or less, or of children under one year of age. This is illustrated in chart 1 after which time the curve for boys is probably higher than that of girls. Up to the height of 80 cm. the metabolism of both

sexes is the same, but boys taller than 80 cm. produce more heat for their height than do girls.

Metabolism of new-born. The physiologic state of the new-born infant is quite different from that of later infancy and will, therefore, be considered separately. The studies of Hasselbalch (54) on new-born infants were obtained under basal conditions. This work, because of its importance, has been completely translated by Miss Alice Johnson and appears with all the tables in Publication 233 of the Carnegie Institution of Washington.

Subsequent work of Benedict and Talbot (16), Bailey and Murlin (6), Murlin, Conklin and Marsh (87), and Marine, Lowe and Cipra (75) substantiate in the main the original findings of Hasselbalch. Interpretation of the findings cannot be intelligently made without bearing in mind the individual physical differences in the new-born infant. Some infants are vigorous, shiver if cold, and cry if hungry, while others are weak, easily chilled and behave passively.

Benedict and Talbot (13) found the lowest heat production, in their series of 105 new-borns, in infants less than twenty-four hours old weighing under three kilograms. This low metabolism occurred more frequently in infants with a subnormal temperature, and may indicate weakness.

There was an increase in basal heat production after the first twenty-four hours as the infant adapted itself to the surroundings, and a slightly higher metabolism, was found approximately 12 per cent, when food was given.

If the metabolism of an individual infant is followed carefully it will seem to be dependent, as is that of the adult, only to a greater degree, upon the length and severity of the initial fast, and the amount and quality of the food taken after the fast is broken.

Murlin (87) found the highest heat production per square meter of body surface in his series of 50 new-borns during the second twenty-four hours, after which it fell gradually and uniformly to the sixth day. No justification for this conclusion can be found in the Benedict and Talbot series of new-borns.

Benedict and Talbot (13) found that a definite relationship existed between the length, weight, and metabolism of a new-born infant. This may be predicted very accurately after the first 36 hours of life by means of the following formula.

$$\text{Total calories} = \frac{L}{} \times 12.65 \times 10.3 \sqrt[3]{wt_2}$$

$\underline{L}$ = length
12.65 = constant
$10.3\sqrt[3]{wt_2}$ = Lissauer's formula for body surface.

The average heat production of the two largest series of studies on new-born infants, Benedict and Talbot's (13) 105 cases, and Murlin's (87) 50 cases is as shown in table 7.

TABLE 7

Average basal heat production of new-born infants

	TOTAL CALORIES PER 24 HOURS	CALORIES PER KILO PER 24 HOURS	CALORIES PER SQUARE METER PER 24 HOURS (LISSAUER)
Benedict and Talbot	95–193	42	612
Murlin	160	48	700

The slightly higher figures obtained by Murlin may be due to larger, heavier babies, or to better nutrition.

Respiratory quotient. A study of the respiratory quotient during the new-born period gives evidence concerning the type of food which is being oxidized during this period of partial starvation. It shows what proportion of fat and carbohydrate is being burned and, consequently, is of value in explaining the physiologic loss of weight. Respiratory quotients during the first 8 days of life are given in table 8.

TABLE 8

Respiratory quotients of new-born infants during first eight days of life

	DAYS OF AGE							
	1	2	3	4	5	6	7	8
Benedict and Talbot (13)	0.80	0.74	0.73	0.75	0.79	0.82	0.81	0.80
Murlin (87)	0.79	0.76	0.75	0.75	0.81	0.80	0.82	0.81

The average post-absorptive respiratory quotient found in normal male adults is 0.81. In the Benedict and Talbot series the respiratory quotient reached that of the normal mixed diet on the sixth day, while in Murlin's series it was reached on the fifth day. Some cases are to be found in each series in which the respiratory quotient of very close to 1.00 on the first day of life indicates that the individual child is burning pure sugar. After a few hours the quotient usually falls and at the end of the first twenty-four hours it is very close to the respiratory quotient of pure fat. In some instances it later went below 0.70 suggesting acidosis. Metabolism and other investigations seem to indicate that the carbo-

hydrate reserve of the body is quickly exhausted at the end of twenty-four hours and that until the mother's milk is secreted in normal amounts the heat energy is obtained in large part from body fat. Individual respiratory quotients bear this out, and the general average of all the cases is consistent with this view. The clearest picture, however, is seen in those infants whose metabolism was done day by day.

From a study of the respiratory quotient of normal new-born infants it may be deduced that the supply of glycogen in the body is quickly used up and that the energy is supplied principally from the body fat until the breast milk "comes in," or until other food is given.

A sub-normal temperature during the first few days of life was found to be greater in the weak infants. It is believed that there is a connection between the low metabolism, the subnormal temperature and the failure to gain in weight. The vigorous infants on the other hand, shiver or cry lustily making enough heat by muscular exercise to replace that lost during the exposure incidental to birth.

Prematurity. The first work on premature infants which meets modern requirements is that of Hasselbalch's (54) which was obtained under basal conditions.

Recent studies have been made by Murlin and Marsh (87) and Talbot, Sisson and others (147), (149), (152).

The basal metabolism of the premature infant is lower than that of the full-term infant, and remains so for at least three months. Even if the handicap of time is allowed, the premature infant produces less heat on his expected birthday than does the full-term infant during the first week of life.

The relation of the total heat production of premature infants to that of normal infants of the same age is shown in chart 3.

These findings show that the total heat production for twenty-four hours of a premature infant is considerably lower but tends to run parallel with that of the normal, full-term infant.

The total heat production of the premature infant has been plotted against body weight in chart 3 which shows also the curve of the heat production of normal, full-term infants. The heavy babies have a greater heat production than the lighter ones.

The total heat production plotted against weight appears to be higher in the premature than in the normal infant. This can be seen only in the heavier infants weighing 2.5 kilograms or more, which can be compared with normal infants.

The total heat production of premature infants is extremely low,

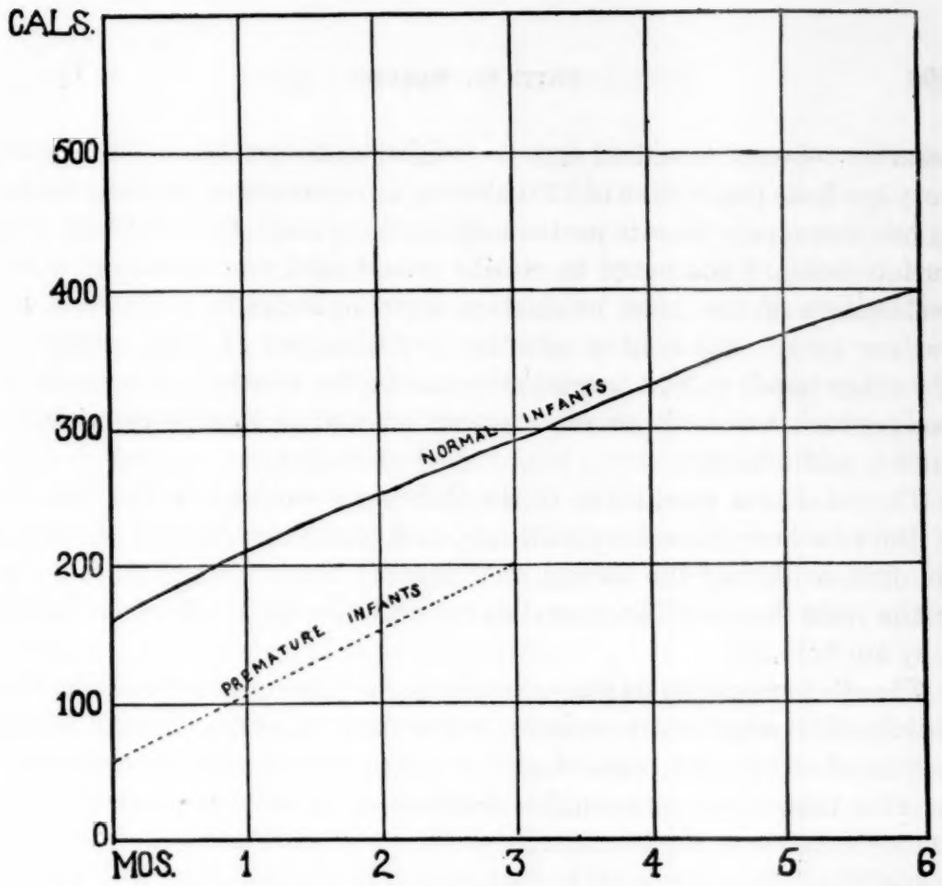

Chart 3

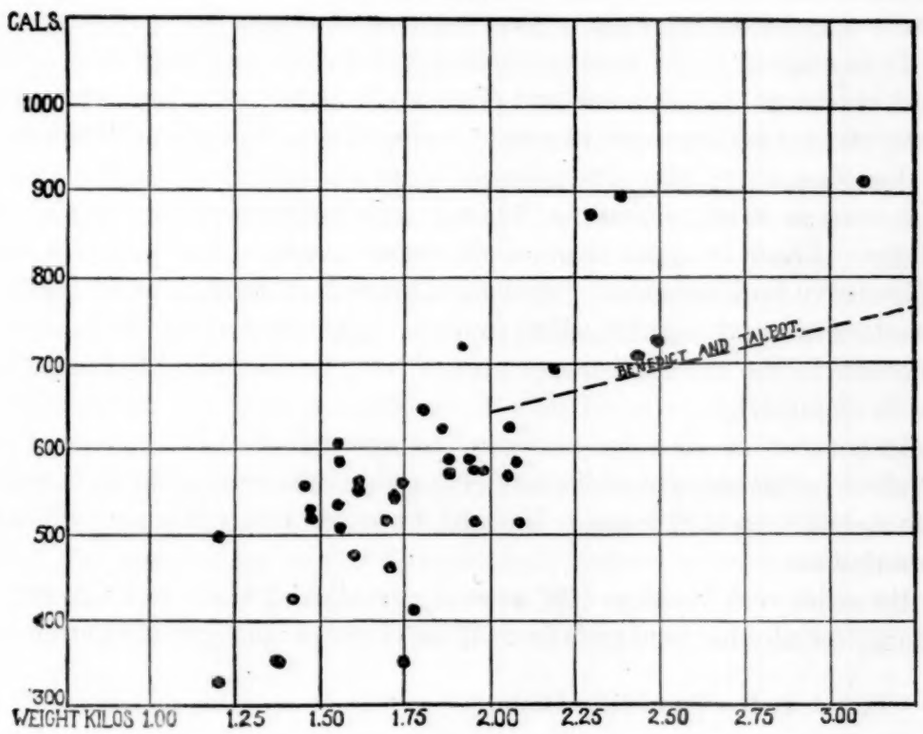

Chart 4

whether referred to actual age, to weight, or to surface. There was a very low heat production of 320 calories per square meter of body surface in one instance. This is particularly striking since the relatively great surface area as compared to weight would lead one to expect a high metabolism if the heat production were universally proportional to surface area. The heat production per kilogram of body weight, on the other hand, is high presumably due to the relative make up of the body which has little or no inactive fat and is largely composed of muscle and organs.

The total heat production of the thriving premature infant, like that of the new-born, increases with age, and sometimes toward the end of the first or during the second year reaches that of the normal infant. If the child does not thrive and develop physically the heat production may lag behind.[3]

The effect of puberty on the metabolism. The period of transition from childhood to adult characteristics is known as puberty. It may roughly be placed at 12 to 14 years of age for girls, and somewhat later in boys. At this time there is a sudden increase in growth associated with a marked change in the conformity of the body, and an awakening of the sexual life. It is conceivable that with this increase in growth and the concomitant change in the activity of the glands of internal secretion, there may be an increase in the basal metabolism.

It is evident from the writings of the earlier investigators, Andral and Gavarret (1), Sonden and Tigerstedt (129) and Olin (95a), that they recognized the onset of puberty played a part in the metabolism.

The first comprehensive piece of work on this phase of the subject was done in 1916 by DuBois (40) who studied boys of 12 and 13 years of age. Later he again studied this same group, after physical signs of puberty had appeared, "under experimental conditions which were practically unchanged." His findings are comparable and show a decrease in the metabolism per unit of body surface after the establishment of puberty.

In a series of six boys between the ages of 12 and 13 years of age studied by Benedict and Talbot (16) no definite increase was found in the metabolism at this age. One girl, however, had a definitely elevated metabolism.

Benedict and Hendry (19) recently published their findings on the energy requirements of girls from 12 to 17 years, and give measurements

[3] Talbot, F. B.: Unpublished data.

taken at night during sleep. They found that the average twenty-four hours heat production per individual of 12 to 17 years of age was 1250 calories irrespective of age; that the calories per kilogram of body weight diminished with increasing age from 29.9 to 21.7 calories, and that the heat production per square meter of body surface diminished from 928 calories at 14 years to 745 calories at 16 years.

Talbot[4] found that if subjects selected from among those who were maturing, had visibly enlarged thyroid glands, there was a distinct tendency for the metabolism to be elevated above normal limits. This probably is a temporary hyperthyroidism of puberty which is not an uncommon clinical phenomenon. If further investigations confirm this, it will explain the accidental findings of a high metabolism at puberty. Such studies can only be made on individuals, as they would be lost in group studies of the metabolism.

More recently the metabolism of girls at this age period has been studied extensively by Benedict (20), Bedale (8), Blunt, Nelson and Oleson (26) and MacLeod (72). There has been considerable discussion as to whether there is a rise in metabolism connected with the prepubescent period. DuBois' studies on Boy Scouts would seem to indicate this. It was not apparent, however, in the few boys studied by Benedict and Talbot. There are more data, on the other hand, about girls, and although obtained in part in the respiratory chamber even during sleep, and in part with the portable apparatus, the added numbers help to fill out the picture. It must be remembered that the Girl Scouts studied during sleep illustrate more strictly minimal periods than do cases whose metabolism was obtained on the portable apparatus.

Benedict (23) after a critical analysis of the data available came to the conclusion that the evidence at hand seemed to indicate that the effect of sleep was negligible; but, on the other hand, that the few Girl Scouts periods available suggested a profound decrease in the metabolism during sleep.

The data of the basal metabolism of girls have been summarized by MacLeod (72). The results can be interpreted to mean either that there is or that there is not a rise of the meatabolism connected with puberty, depending upon which method of study is made. If the total calories are plotted against age on the Benedict and Talbot curve, it is apparent that there is a very definite increase in the metabolism commencing with the eleventh year, reaching its peak in the fourteenth and fifteenth

[4] Unpublished data.

years. If this increase in metabolism be compared with the curves of yearly growth it will be seen that the increase in growth at puberty coincides in general with the increase of metabolism of the age. This seems to indicate that there is a greater relationship between physical development than age development. If, however, the total calories are plotted against weight, there is no such specific impulse to the metabolism apparent and it is probable that the same is also true when the total metabolism is plotted against height. This seems to indicate that the apparent increase in the rate of the metabolism seen in total calories referred to age, and calories per square meter referred to either age or weight, is the result of very rapid growth and may be explained by the greater size of the child rather than any specific change in the metabolism associated with puberty.

Standards of basal metabolism. Until the Benedict and Talbot series of normal boys and girls was published, there was no normal standard with which to compare and interpret the metabolism of pathological cases. In order to establish the normal basal metabolism of infants and children, Benedict and Talbot (16) made a series of investigations covering a period of over ten years.

Recently these normal figures have been supplemented and the age period increased up to 17 years by Benedict and Hendry (19), (20), Benedict (22), Blunt (26), MacLeod (72), Bedale (8) and Takahira.[5]

The metabolism of the new-born infant, that is during the first few days of life, may be very closely computed by the formula of Benedict and Talbot:

$$\text{Total calories} = L \times 12.65 \times 10.3 \sqrt[3]{\text{wt}_2}$$

In this formula L = length
12.65 = constant
$10.3\sqrt[3]{\text{wt}_2}$ = Lissauer's formula for body surface

Murlin's new series falls somewhat higher.

The metabolism of girls from one week to 12 years may be predicted according to height alone as recommended by Benedict (23). This is open to less error than prediction from body weight.

Benedict and Talbot (16) found the total metabolism referred to weight predicts very closely the metabolism of boys from 2 weeks to

[5] Prof. Hideo Takahira recently sent me unpublished data of 71 Japanese children, 56 boys and 15 girls, between the ages of 6 and 19 years whose basal metabolism was obtained in the cot chamber. A superficial analysis of the data shows that the Japanese children conform roughly with the American standard.

12 years. Talbot also found that the heat production of normal boys may be predicted from height alone as in girls (see tables 5 and 6).

Talbot (145) has recently published curves of the basal metabolism according to age, weight, and surface making allowance for a 10 per cent variation on either side of the average curve. He emphasizes the importance of estimating the physical development of these children and with this in view has published other curves (157) of the physical measurements of the children whose metabolism go to make up the standard curves.

These growth curves are of especial interest in basal metabolism studies, because they differ from the growth curves obtained by others in foreign countries and parts of this country, and also from children of a different social status in the same city. Children of the well-to-do are taller and heavier than those of the less fortunate grades of society. The physical development of a child can be placed according to the curves mentioned above, and can be taken into consideration in the interpretation of its metabolism.

Many writers, on the other hand, prefer to use heat production per unit of body surface as the sole method of comparison. While this may be accurate for the adult, the writer has found many unexplainable variations on this basis in pathological cases especially where infantile malnutrition is a complication. (See section on the metabolism of malnutrition.)

Although there has not been sufficient opportunity to test out the value of the total heat production against height in pathological cases, it has been the most satisfactory single method proposed so far for normal children. The writer feels, however, that until there is a greater accumulation of pathological material, it is better to compare the heat production with all the standards in order to obtain a completely rounded out picture. At the present moment it seems probable that the total metabolism for height will be the best single standard for both girls and boys, from 2 weeks to 12 years.

The twenty-four hour metabolism. A review of the literature shows that there have been few instances in which an attempt has been made to obtain the twenty-four hour metabolism of normal infants. The fundamental experiments of Rubner and Heubner (102), (103) stand out as giving the first twenty-four hour figures available for normal infants.

Talbot (138) has reported two cases in which the metabolism was studied over twenty-four hours in half-hour periods. The active

periods could then be separated from and compared with basal periods. In one case the active periods produced an average of 20 per cent and in another of 30 per cent more calories than the basal periods.

Studies of the metabolism of infants over twenty-four hour periods have to be interrupted for feedings, but the infant respiratory chamber is so constructed to make possible the shortest loss of time. Indeed in one of the cases reported above, the metabolism was obtained for 23 hours and 10 minutes out of the twenty-four hours, leaving only 50 minutes to be estimated. The older apparatus is not constructed to give such detail of data, nor such continuous observation as the respiratory chamber. More studies of this type of both the normal and abnormal infant are desirable because from the data obtained can be deduced much information of value to the clinician.

Influence of muscular activity on the basal metabolism. The influence of muscular activity on the basal metabolism has been recognized since 1898 when Rubner and Heubner observed that a restless infant produced more heat than a quiet infant.

Howland in 1913 (60) emphasized the fact that the "effect of active exercise upon metabolism, even that of a small infant, is very great." He felt that comparable conditions were only obtainable during sleep.

Although Schlossmann made visual estimates of the amount of muscular activity, it was not until graphic records and frequent counts of the pulse were made by Benedict and Talbot (12) that it was possible to obtain a quantitative estimation of the amount of activity which was more than subjective.

Benedict and Talbot recorded graphic measurements of the muscular activity on the smoked drum of a kymograph. The bed upon which the subject lay was so arranged that slight movements of the patient changed the position of the bed, and this activity was communicated by a Porter pneumograph, or similar contrivance, to a pointer which recorded it on the revolving smoked drum of the kymograph. When the bed was carefully adjusted to the weight of the subject such slight movements as those due to respiration could often be detected on the smoked drum. A quiet basal period was indicated by an unbroken horizontal line. A full description of this method is given in Publication 201 of the Carnegie Institution of Washington.

The kymograph record and records of the pulse rate, taken at the same time by means of a stethoscope attached to the infant's chest, gave very accurate estimations of the muscular activity. Muscular activity may also be detected from sounds coming through the stetho-

scope which makes it possible for the observer to estimate by the ear as well as the eye what the infant is doing.

Talbot (138), on the basis of the twenty-four hour observations mentioned above, concluded that a rough estimate of the total caloric requirements of a normal infant may be made. In a very quiet infant 15 per cent may be added to the basal metabolism for activity, if normally active 25 per cent, and if extremely active about 40 per cent. In one new-born infant Benedict and Talbot found an increase of 211 per cent in a short time due to crying (12). Murlin (87) goes so far as to say that crying one per cent of the time raises the metabolism one per cent. The difficulty of obtaining a true measure of the work done during muscular exercise makes it impossible to more than estimate how much varying amounts of exercise affect the metabolism.

The effect of ultraviolet ray on the basal metabolism. This has been studied by Fries (52) in Schick's Clinic and although detailed protocols are not available, the results in the few cases studied seem to indicate that ultraviolet light does not cause any prolonged rise in the basal metabolism. More work must be done on this problem, at varying intervals after exposure, before a final conclusion can be reached.

II. Basal metabolism in abnormal conditions and disease. *Malnutrition.*[6] Although studies of the metabolism of numerous cases of infantile malnutrition were recorded by Benedict and Talbot (12), no comprehensive study of malnutrition as a clinical entity was made until Fleming (44) reported his series, and Talbot (146) later reported an additional series. These as well as the cases studied by Murlin and Hoobler (82) and Howland (60), and the cases of malnutrition in older children studied by Blunt, Nelson and Oleson (26) are comparable since the metabolism in all these studies was obtained under basal conditions.

Fleming (44) and later Talbot's studies (146) on the basal metabolism of atrophic infants showed the necessity for giving additional food calories to these infants in order to obtain an increase in weight. The more an infant is under weight the greater are its caloric needs per kilo for growth. The basis of this is shown in chart 5, which gives the basal heat production of infants with different grades of malnutrition.

The shaded area between the two dash lines represents the limits of heat production of normal infants.

[6] References to the earlier European work on atrophic or malnourished infants may be found in the bibliography. They will not be discussed here since conditions are not comparable due to the technic used.

Fleming (44) concluded that until an infant becomes 35 per cent under weight there is no fundamental change in the metabolism. On the basis of chart 5 Talbot (146) concluded that an infant could be about

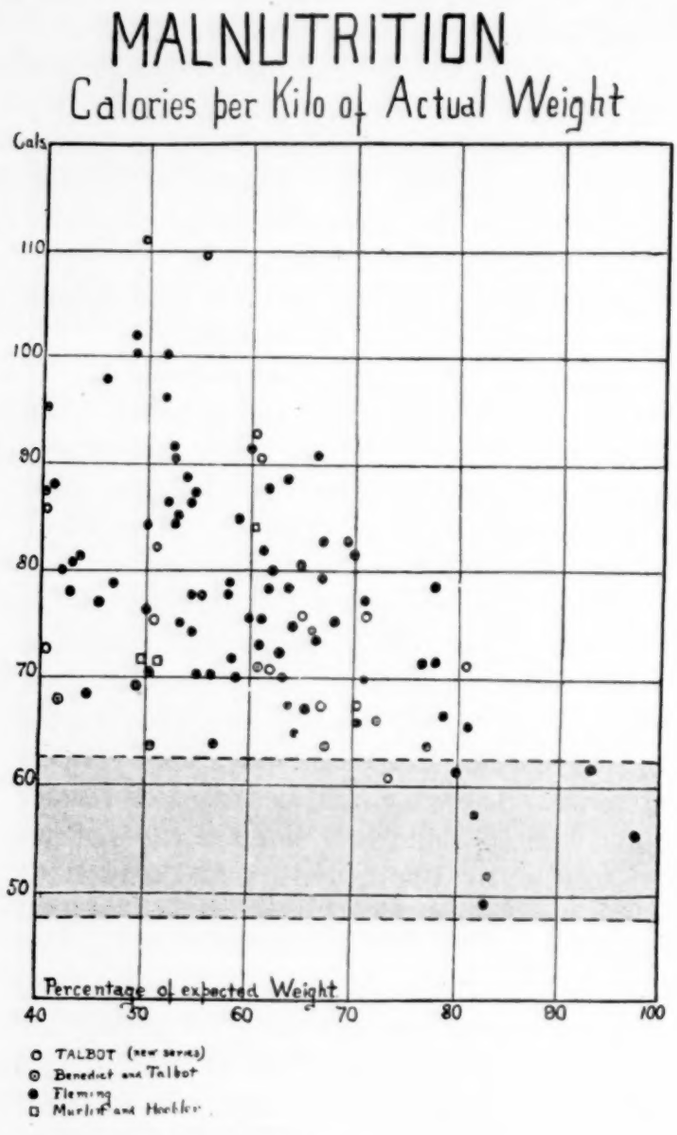

Chart 5

20 per cent below the average weight for the age before the calories per kilo were higher than the upper normal limit of 62 calories.

This chart shows the more an infant is under the average weight the more calories it produces per kilogram of body weight. The conclusion is also reached that the relation of active to inactive metabolic tissue has

changed and that the more underweight the infant is the less inactive tissue there will be as compared to the active tissue. This statement must be qualified, however, because in the most severe grades of malnutrition there is presumably also less active tissue, muscles and organs than in the normal infant of the same age and height.

Undernutrition is not of necessity at the expense of inactive tissues alone; a comparison of the total heat production of these infants with that of infants of the same age illustrates this. Chart 6 shows how the total metabolism may be lower than the expected metabolism for the age.

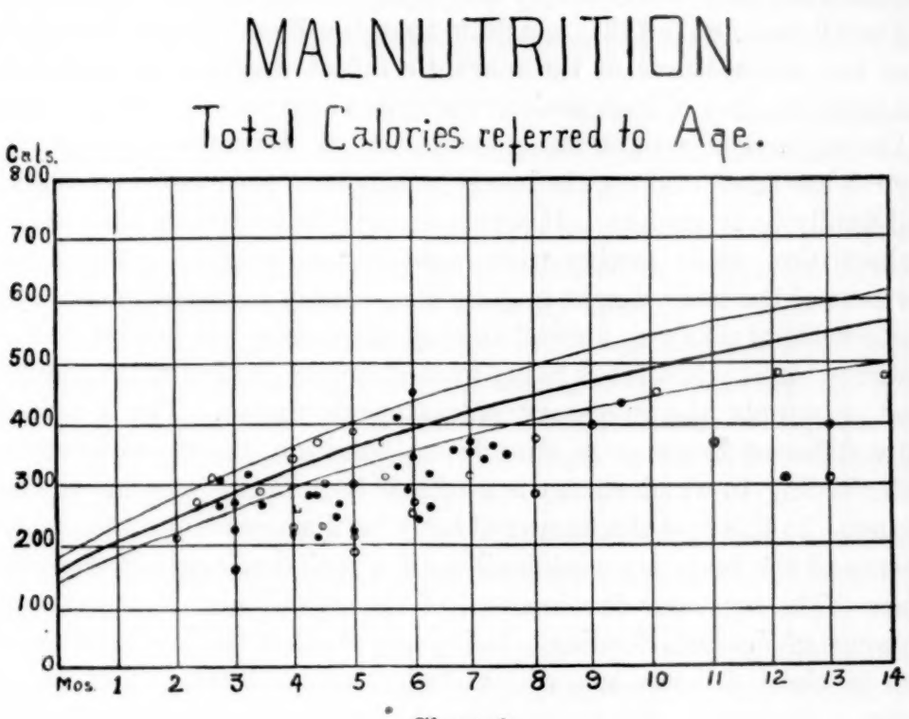

Chart 6

The total heat production of a large proportion of these infants was distinctly less than that of normal children of the same age. This would seem to indicate that there is less active protoplasmic tissue (muscle and organs) in the bodies of these infants than in the normal infant of the same age. The metabolism of underweight older children gives no evidence to discredit the presumption that the change in the metabolism is due primarily to absence of body fat.

Blunt, Nelson and Oleson (26) showed an excess metabolism per kilo and per square meter of body surface as compared to the Benedict and

Talbot normals; in some cases it was plus 40 per cent. They were unable to find any relationship between the percentage underweight and the excess metabolism, but with the exception of four cases, all were less than 20 per cent underweight; the greatest deviation from the normal weight was 27 per cent. The variations were, therefore, much less than among infants the great majority of whom were more than 30 per cent underweight and some of them 50 per cent. The older children, therefore, did not show such severe grades of malnutrition as did the infants.

Fleming and Hutchinson (48) conclude that the disturbance of metabolism in marasmus is simply the result of inanition and not the cause.

Tisdall and Drake (161) conclude from the Fleming and Talbot data that the active tissue of the marantic infant produces approximately the same amount of heat as does the active tissue of the normal infant.

During loss of weight the presumption is, that after the glycogen reserve has been used up, the loss is primarily at the expense of body fat and finally body protein. It seems reasonable to assume that in acute malnutrition, as in fasting with rapid loss in weight, as much as 30 per cent of the body weight may be lost, mostly in the form of inactive tissue without using up a great amount of protein. It has been shown, however,[7] that when fat is being burned during fasting some protein is used to supply the necessary antiketogenic factor. The problem is quite different however in chronic malnutrition due to indigestion or underfeeding, in which energy is available both in the food and the body tissues. In this case the reserve of body fat disappears and the glycogen reserve of the body is maintained until a period of acute fasting intervenes. The muscular development of the child is also retarded in consequence of the underfeeding. It is probable that the low total calories seen in chart 5 indicate a diminished amount of active protoplasmic tissue in the body. No practical method is yet available by which the total amount of muscle in the living body can be measured. A further possible explanation of the low metabolism is, that the amount of muscle is normal but that its tone is subnormal, thus resulting in a lowered metabolism. The evidence at hand is in favor of the former explanation.

The importance of knowing how the metabolism is affected by malnutrition cannot be over-emphasized when pathology is studied, because pathological cases are so frequently accompanied by some degree of

[7] Shaw, E. B. and M. E. Moriarity. Hypoglycemia and Acidosis in Fasting Children with Idiopathic Epilepsy. Amer. Journ. Dis. Child., 1924, xxviii, 553.

either acute or chronic malnutrition, which must be taken into consideration in the interpretation of results.

Fasting. Benedict and Talbot (12) and Schlossmann and Murschhauser (122) studied the fasting metabolism of infants for twenty-four or more hours but were unable to get sufficient basal data for comparison to make any far-reaching conclusions.

Talbot, Hendry and Moriarty (156) recently studied the basal metabolism of children undergoing therapeutic fasts for the treatment of epilepsy and obtained figures similar to those reported by Benedict and others for fasting adults. The total metabolism, skin temperature and pulse rate were found to decrease as the period of fast progressed. It is not yet clear how this is affected by the compensated acidosis which accompanied the fasts.

Hyperthyroidism. The fact that the basal metabolism was increased in exophthalmic goiter and decreased in cretinism and myxedema was first established in 1895 by Magnus-Levy (73a).

Hyperthyroidism is much less common in childhood than hypothyroidism and fewer reports on basal metabolism studies in hyperthyroidism in children are recorded in the literature.

Talbot (150) in 1922 reported a case with a plus 34 per cent basal metabolism, which fell after the ninth roentgen ray treatment to plus 26 per cent. From what data are available it seems reasonable to conclude that the same principles which apply to hyperthyroidism in the adult are also applicable to the child.

Non-toxic goiter. Reports of basal metabolism studies in non-toxic goiter by H'Doubler (55) and Talbot (150) were found to be normal as in the non-toxic goiters of adults.

Cretinism. Bergmann in 1909 (24) reported the metabolism of a 15-month-old infant with infantile myxedema and found a minus 29 per cent metabolism.

The first American study on the basal metabolism in cretinism was made in 1914 by DuBois (37) who reported that the metabolism of a 36-year-old cretin with a mentality of an 8-year-old child was from 18 to 25 per cent below normal. After three days of thyroid therapy he was able to raise the metabolism to normal.

Talbot in 1916 (137) found the metabolism of a $3\frac{2}{3}$-year-old cretin to be 20 per cent below normal.

Fleming (45) has recently reported his observations on two cretins, one aged 23 months and the other 4 years and 8 months before and during treatment. He also found the basal metabolism of an untreated

cretin much below normal and was able to obtain an increase in the metabolism by the administration of thyroid extract.

Janet in 1923 (62) reported the metabolism of two cretins one of which was minus 26 per cent and the other minus 48 per cent.

Subsequent studies by Talbot on the metabolism of twelve cretins (151), (153) before and during treatment showed that thyroid therapy raised the metabolism of cretins, and that omission of treatment resulted in a lowered metabolism. The conclusion was reached that the dosage should be so regulated that the basal metabolism is brought up to the expected total metabolism for the age before the best results are obtained. If this view is accepted, the use of the metabolism per unit of body surface or body weight will lead to erroneous interpretations of the metabolism of children with cretinism.

The signs of sporadic cretinism probably develop after birth in many instances but have been recognized at birth. The physical characteristics of the disease are not usually recognized until the sixth or eighth month of life, but may be recognized clinically as early as the third month. The basal metabolism is lowered at this time and possibly earlier and consequently is useful in determining the diagnosis in uncertain cases. The lowered metabolism and symptoms due to deficiency in thyroid secretion may be corrected by thyroid therapy. The failure to recognize and treat cretinism at the third month or earlier is probably responsible for many of the past failures in obtaining normal mental development in cretins.

Myxedema. Metabolism studies in myxedema during childhood have only been reported in one instance (150). The metabolism in this case which was minus 20 per cent was brought up to normal by the administration of thyroid extract.

Mongolian idiocy. Fleming (45) in a recent report on a series of Mongolian idiots concluded that their basal metabolism was normal, and consequently that there was no indication for thyroid therapy.

A series of twenty Mongolian idiots has been under observation at the Massachusetts General Hospital over a period of four years.[8] Although the basal metabolism findings were apparently normal in some instances, and low in others, he concluded that the older the subject the more subnormal the metabolism. Most of these children showed definite temporary mental and physical improvement under thyroid therapy.

[8] Talbot, F. B. Unpublished data.

Diabetes insipidus. Christian (35) with the help of Miss Tompkins found a low metabolism in a five-year-old girl with diabetes insipidus. These findings applied to the Benedict and Talbot standards showed a metabolism of plus 27 per cent.

A case of diabetes insipidus[8] in the Children's Clinic of the Massachusetts General Hospital had a normal metabolism.

Diabetes mellitus. Benedict and Joslin (9) found an increase in the total heat production in cases of severe diabetes with acidosis. These studies do not include the basal metabolism of young children.

Talbot (150) mentions the metabolism of two cases in a series of eight children studied before the advent of insulin. The caloric intake of one barely covered the basal metabolism and the child lost weight continuously, while in the other the caloric intake was greater than the basal needs leaving enough food for exercise and growth as shown by the increasing weight.

Dwarfism. The metabolism of a 7-year-old dwarf (144) was found low for the age but the intensity of the metabolism was high as shown by the fact that the total metabolism per kilogram of body weight, and per square meter of body surface were high, being 20 per cent or more above the average metabolism. The child's metabolism findings are similar to those of infants with severe infantile malnutrition.

Metabolism of a case with congenital absence of the cerebral hemispheres. A case of an infant with congenital absence of the cerebral hemispheres was studied in 1915 (135). This case was first seen at 8 months of age. He was unable to sit up or hold up his head, and there was an idiotic expression of the face. Physically he was a large baby with a thick layer of subcutaneous fat. An operation for relief of blindness showed that the hemispheres of the brain were entirely absent and were replaced by cerebrospinal fluid.

His metabolism was low. This seemed to be due to the fact that clinically the child's body was composed of a large amount of fat and a relatively small amount of muscle.

Metabolism of a case of dyspituitarism. The metabolism of a very obese child with a small sella turcica resembling the type of dyspituitarism described by Frölich (143) was determined, at the age of 2 years and 9 months in a child weighing 24.82 kilos. The basal metabolism was low. Clinically much benefit was derived from the administration of thyroid in a dosage of 6 grains a day.

Amaurotic family idiocy. The metabolism of a typical case of amaurotic family idiocy with malnutrition was studied in 1918 and

found to be low (140). There was a low pulse rate which seemed to indicate a relationship between the metabolism and the pulse rate. Since there was no subcutaneous fat, or any excess of water in the tissues, the low metabolism must have meant either that the muscles were diminished in amount or did not have a normal tone. A second case of amaurotic family idiocy, B. L., (as yet unpublished) an infant 19 months old, showed a minus 15 per cent basal metabolism. Further investigations of this disease are necessary before concluding that a low metabolism is characteristic.

Congenital stenosis of bile ducts. Fleming in 1922 (46) found the metabolism considerably raised in an infant of 4 months with congenital stenosis of the bile ducts.

Progeria. The metabolism was normal in a 12-year-old girl with progeria (154).

Idiopathic convulsions (epilepsy). In view of the reports which appear in the literature from time to time on the use of thyroid in the treatment of epilepsy, studies of the basal metabolism of eleven epileptic children were made (156). It was found that the basal metabolism of this series was either normal or elevated. This was interpreted to mean that thyroid gland medication is not indicated in the treatment of idiopathic epilepsy of childhood.

Ichthyosis. The basal metabolism of children with ichthyosis (158) was found to be normal or elevated in most instances, although M. Krogh found it minus 20 per cent in a woman of 29 years. As the majority of cases in the literature do not have a subnormal metabolism, the conclusion has been reached that ichthyosis is not due to or related to hypothyroidism.

There are many physiological problems of the energy metabolism of childhood which still remain unsolved, and since the field of pathology has been barely scratched there is much to be gleaned from it. Although some problems have been touched upon there still remain many questions to be answered and it is to be hoped that, with the increasing number of investigators in the field, data which have accumulated so slowly and laboriously in the past will increase much more rapidy in the future.

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REVIEW ON THE TONUS OF SKELETAL MUSCLE

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There is need to restate the evidence upon which rests our present explanation of tonic muscular contraction. A survey of the recent journals reveals many speculative papers on tonus which lead to unwarranted conclusions. Speculation may be useful as a stimulus to thought; but in these days of facilitated authorship, mere repetition of a theory often leads to its uncritical acceptance.

To review adequately the whole subject of tone is beyond the scope of a single paper. Moreover Sherrington in 1915 (126) and Spiegel in 1923 (131) have given us excellent general reviews.[1] Here then will be considered only the problem of tonic innervation of skeletal muscle. Some reference will of course have to be made to analogous conditions such as contracture. This is allied to tonic contraction, but cannot here be discussed for it would take us far afield to study muscular shortening due to drugs,[2] toxins and physical changes; these are of interest to our problem and certainly related, but must wait until further evidence shows where the relationships are to be looked for. For recent work along these lines which may eventually throw light on the problem of muscle tone, the reader is referred to papers by Gasser and Hill (53), Meyerhoff (94) and Frank (49).

Clear definition of the problem is important. The most specific description of the field with which this paper is concerned can be found in Sherrington's exposition on "Postural Activity of Muscle and Nerve" (126) where he says "Reflex tonus is in short reflex posture" . . . . "a reflex which differs from the reflexes more commonly studied mainly in this, that the latter execute *movements* while this maintains *posture*."

We do not deal then with slow movements, with rhythmic contractions, with cramps, spasms or contractures—but with that *state of*

[1] For the historical side of the subject, and for a full bibliography up to 1923, the reader is referred to Spiegel's excellent monograph.

[2] Adrian (3) says: "We can put little reliance on the analogy between veratrine contracture and the physiological state of tone."

constant muscular tension which underlies and makes possible all orderly motion. Thus muscle itself must first receive attention, then its innervation, and finally the mechanisms by which the central nervous system builds up tonic reflexes of different degrees of complexity.

I. THE STRUCTURE OF MUSCLE, ITS INNERVATION AND TYPES OF CONTRACTION. Although tonus is not an inherent property of striated muscle, as it is of smooth muscle, one must review *the anatomy of muscle* carefully in order to understand the possible effects of the nerve impulse upon it. Even in this field one is surprised to find indefiniteness, partly because the classification of the different types of muscle is inherently difficult on account of intergradations and transitional forms; partly because the histology of muscle has often been overlooked in the study of its physiology. In fact it seems that no sharp lines can be drawn between the different accepted types of muscle. *Smooth muscle and striated muscle* seem different enough, but when one considers the muscles of the eye-ball, larynx, tongue, diaphragm, and heart[3] it is difficult to see where to draw the dividing line between the two obviously different extremes. In fact Piersol (108) states that no fundamental difference can be drawn between them on purely morphological grounds. Bryce (23) believes that while there is a distinction it is not absolute, smooth muscle representing a less advanced stage of the striped variety.

Red and white muscle. Having pointed out that smooth and striated muscle are not sharply distinguishable, it is necessary to take up striated muscle more in detail. The fact that there are two types of muscle fibre—the red and the white—in striated skeletal muscle is generally known, and has been accepted since the work of Ranvier (112). Good descriptions of these differences are given by Schafer (125), Starling (132) and Hay (61).

The question of the distribution of red and white muscle has been studied by several authors (112), (10), (57) but there is no unanimity of opinion and since most of the observations are gross, backed with little or no histology, one can attach little importance to the discussions of white pectoral muscles in the fluttering fowl as compared with

[3] The heart muscle is obviously intermediate between smooth and striated muscle; several muscles of the eye-ball are mixed; and the muscles to the larynx, tongue and diaphragm are innervated by what Gaskell (52) calls non-ganglionated splanchnic efferents. Therefore, it is probably improper to apply directly to skeletal muscle evidence gained from these muscles, as has been done by some investigators (73).

red in the soaring buzzard (10), especially as the many exceptions to the rule are not explained. Starling's (132) view is probably the correct one—that all striated muscle of higher vertebrates is mixed, although certain small muscles may be almost entirely red or white. The distribution is not always the same, however, even in the same genus or species, for muscles which are white in the domestic rabbit may be quite red in a wild rabbit (57), while in man, the muscles of an athlete in good condition are distinctly redder than those of a senile or "soft" individual. Moreover, Morpurgo (95) has shown that the hypertrophy of muscle following exercise is not due to an increase in the number of striated fibres, but to a filling out of the already existing elements with more sarcoplasm.

It is apparent, therefore, that the anatomical factors have not been thoroughly studied and coördinated. Probably the redness or paleness of a muscle is not a fundamental feature. Rather should we use the histological criteria, and divide muscles into a primitive type, with small fibres and centrally placed nuclei, and a more highly developed type with larger fibres, less sarcoplasm, clearer striations, and peripherally placed nuclei. In general, but probably not always, the primitive type contains more pigment, and is, therefore, redder than the larger striated fibres. It must be emphasized here, however, that the state of knowledge concerning the red and white muscle fibres is at present so confused, even in the field of anatomy, that much work must be done before physiological interpretations can be acceptable.

The work of Ranvier (112), (113) and Grutzner (58) over thirty years ago showed that red muscles contract more slowly than white, and after a longer latent period. It was also observed that red muscle when stimulated with rapidly repeated induction shocks fuses its contraction into a smooth tetanus at a lower stimulation rate than does white muscle. There is very little recent work on the subject, but Adrian (3) states that the electrical response of red muscle has the usual form, though slower in development and decline, than white muscle. Cobb and Fulton (30) have shown that the electrical response of red muscle is slower than that of white, and comes after a slightly longer latent period. Mechanical twitches were registered by isometric lever and optically recorded; they essentially substantiate the earlier work as to duration and summation, and show in addition a very prolonged flat top in the red muscle curve. Thus, although red muscle contracts more slowly and after a longer latent period than

white muscle (76), it must not be thought that its contraction in any way resembles that of smooth muscle (51). Several recent authors have inferred this, and used it as an argument in favor of the sympathetic innervation of striated muscle.

The muscle spindle. The third element in striated muscle—the spindle with its intrafusal fibres—has since Sherrington's (127) researches in 1894, been recognised as an organ with a specific sensory function. He both corroborated the histological work of Ruffini (121), (122) and proved that the latter's speculation as to the spindle's sensory function was indeed correct. Both these authors describe two kinds of nerve endings in the spindle: the large ribbon-like sensory ending connected with the coarse myelinated nerve fibre, and the fine "terminaison en grappe" with its delicate unmyelinated nerve. Kulchitsky (77) in 1925 fully substantiated their anatomical findings and illustrated both types of innervation with his drawings. Sherrington also carried out degeneration experiments, cutting the sciatic nerve: he found that all the muscle fibres except those within spindles were degenerated almost beyond recognition, but that the spindle showed no degeneration even after many months and their intrafusal striated fibres appeared normal. He describes these intrafusal fibres as histologically similar to red muscle fibres (128). The great contribution of Sherrington's papers, however, is the proof that muscles are amply supplied with sense organs, from one-half to one-third of the bulk of a so-called "motor" nerve to a muscle being made up of sensory fibres arising from cells in the dorsal root ganglia.

These observations are fundamental to an understanding of muscle tone. In the first place they show us the afferent side of the reflex arc—the path of the proprioceptive impulses—on which depends the recently described stretch-reflex (87), and ultimately all tonic reflexes. Secondly, they indicate that certain striated muscle fibres of the red variety are innervated by fine non-myelinated nerves. These may be sympathetic in origin but Sherrington refrains from speculation on on this point. Kulchitsky, however, expressed the opinion that these nerves to intrafusal fibres were motor and sympathetic; and Elliot Smith illustrates the mechanism with his diagram (65) published in Hunter's lectures. Boeke (12) believes these endings to be sensory. Dart (34) suggests that there is a somewhat independent peripheral reflex mechanism with ganglion cells; Kulchitsky (77) has found a few such peripherally situated cells, but Agduhr's experiments (5) indicate that the cells of the sympathetic fibres lie in the ganglia of the sympathetic chain.

The innervation of striated muscle. The discussion of nerve-endings in muscle fibres is a question so bound up in the intricacies of special technique that only a brief summary of results should be attempted by any one not an expert in that highly specialized field. For a thorough discussion, therefore, the reader is referred to the excellent reviews by Wilson (144) and Langley (85). A study of the literature indicates that there are certain things which we may accept as proved: 1, that myelinated, somatic, motor nerves end in motor end-plates in some striated muscle fibres; 2, that somatic sensory nerves go to the muscle spindles; 3, that non-myelinated nerve fibres have still other endings on some striated muscle fibres.

Many points in this discussion of muscular nerve-endings are still unsettled and controversial. Those which seem more particularly pertinent to the subject of tonus are: 1, Which fibres are innervated by cerebro-spinal nerves, and which, if any, by sympathetic nerves? 2, Is there a difference in innervation between red fibres and white fibres? 3, Is the innervation of any one muscle fibre single or double?[4] 4, Can sarcoplasm be considered a contractile substance?

1. The question of sympathetic innervation. That there is a sympathetic innervation of striated muscle fibres is still gainsaid by some physiologists. Since the original observations of Tchiriew (135) in 1897 the evidence has slowly accumulated until the more recent work of Boeke (15) and his collaborators, especially Dusser de Barenne (17) and Agduhr (5) has practically proved the point. Still Langley (84) is most cautious in accepting the results of Boeke, for with our imperfect staining methods he believes it easy to mistake blood-vessel nerve endings for muscular, even endomuscular, terminations. Besides it is impossible to say that if the non-myelinated fibres could be traced back far enough in a preparation, they might not be found to arise from myelinated trunks. Boeke and Bielschowsky however, both point out that it is not only the lack of myelin that distinguishes the sympathetic fibres, but their small calibre, peculiar structure, fine endings and distribution in loose plexuses—all in contrast to the somatic nerves and their non-myelinated terminal portions.

Boeke's (13), (14) earlier experiments were suggestive but not con-

[4] As Wilson points out, the term "double innervation" is not a good one for it might refer to the plurisegmental innervation of a single muscle fibre through somatic nerves, as well as to the double innervation of a muscle by both somatic and sympathetic fibres. It is only in this latter sense that the term is here employed.

vincing, since, as Wilson (144) says, too much of the validity of these experiments depended on differences in the degeneration time; and since no stringent proof was brought forward that true sympathetic fibres reached the end organs. In 1919, however, Boeke and Dusser de Barenne (17) conducted a new series of experiments which gave better evidence. They cut the anterior and posterior roots of the 6th to 9th thoracic nerves and extirpated the corresponding spinal ganglia. After 30 days' degeneration the animals were killed and the appropriate intercostal muscles were examined. No myelinated nerves, no motor end-plates and no sensory end organs were found, but the muscles were still plentifully supplied with non-myelinated fibres and "terminaisons en grappe." Good illustrations are shown of these changes.

In the same year Agduhr (6) performed similar experiments on the interosseus muscles of cats' paws by cutting the spinal nerves between the root ganglia and the point of divergence of the white rami communicantes (see fig. 1, $D + E$). Five to ten days later Bielschowsky preparations were made of the muscles involved, and these showed complete degeneration of all motor and sensory medullated nerves. Many non-medullated nerves, however, were found to be still intact; these lay partly along the remnants of the spinal nerves and partly along blood vessels. Some of them could be traced to muscle fibres or to the fibres of the muscle spindles. The recent work by Kuntz and Kerper substantiates these results (79). Thus is confirmed Boeke's theory that the "accessory" endings are sympathetic. Agduhr (5), however, did more than this; he performed the converse experiment, that is, he removed the ganglion stellatum of the sympathetic trunk and, after six days' degeneration, found in Bielschowsky preparations of the interossei many degenerated non-medullated fibres. Dusser de Barenne (37) has found the same thing; after removing the sympathetic abdominal chain the sympathetic endings in the hind leg muscles are degenerated.

These then are the principal pieces of work which support the theory of the sympathetic innervation of striated muscle. Wilson reviewing the subject in 1921 concludes that these experiments have settled beyond reasonable doubt the sympathetic origin of the "accessory" innervation. He states that the morphology indicates centrifugal conduction, presumably motor, and possibly tonic. Since then, Kulchitsky (77) has published an anatomical paper describing the nerve endings in the muscles of the python. He used the method of teasing

muscle in order to show more of a single fibre in each preparation than can be seen by the method of making thin sections, as done by Boeke and others who used the Bielschowsky stains. He shows terminations "en grappe" on the finer muscle fibres and ordinary motor end-plates on the thicker fibres; the former receiving fine, non-medullated nerves

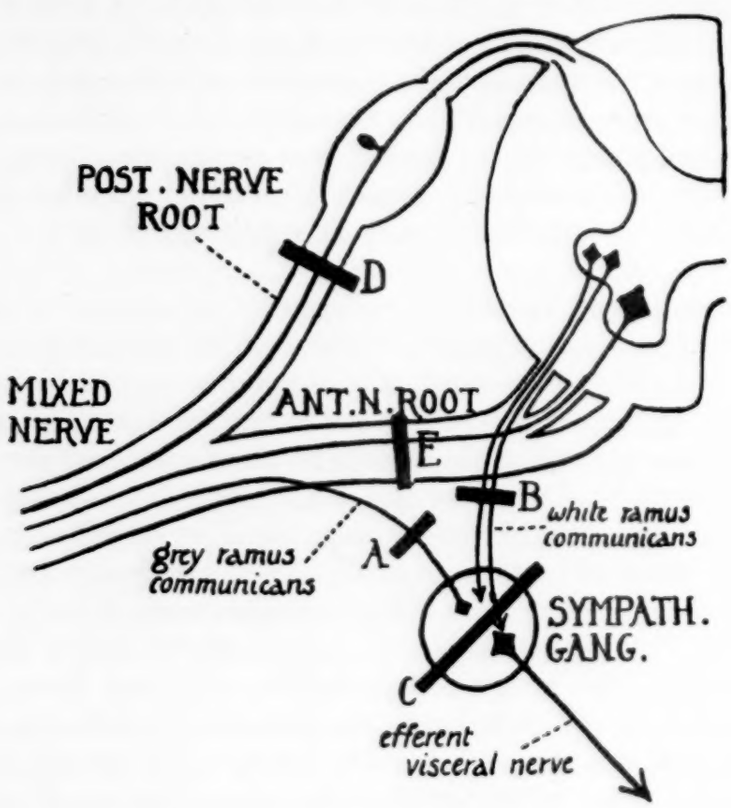

Fig. 1. Diagram of the essential sympathetic connections of the cord and nerve roots (after Hunter (65)). The letters indicate the possible loci of operative lesions:

A = the "ramisection" of Royle.
B = cutting the preganglionic fibre.
C = evulsion of the ganglion of the sympathetic chain.
D = de-afferentation.
E = section of the motor fibres leaving the sympathetic supply intact.

and the latter very much thicker, medullated nerves. He expresses the opinion that these terminations "en grappe" belong to the motor sphere and thinks it quite possible that the non-medullated fibres belong to the sympathetic system. Another paper published this year by Hunter (posthumously) and Latham (69) describes the striated

muscle of the goat and hen as having a rich supply of peculiar nerve-endings derived from plexuses of non-medullated, nucleated, sympathetic nerves. These endings are quite distinct from the ordinary motor-end-plate. Their paper is not well illustrated and appears to be merely a preliminary report.

Bielschowsky (11) believes that the question of double innervation is still obscure in many ways and needs further study especially from the quantitative point of view. He finds that the end organs described by Boeke are most numerous in the external ocular muscles, less numerous in the facial muscles, and very occasional in the muscles of the extremities. He considers it proved that these are sympathetic nerve endings, saying that the fibres may be recognized by the fact that they are non-medullated throughout their whole course, whereas the cerebro-spinal fibres only become non-medullated shortly before their junction with the end-plates.

Lastly Tello (136) studying the embryological development of nerve endings in muscle, finds a distinct evolution for each of the three types of ending—the ordinary motor end-plate, its occasional ultraterminal ending, and the "accessory" termination of Boeke and Perroncito. All these are met with in the embryonic muscle.

Thus, although the subject is far from settled, we can say with Langley (84): "There is a balance of histological evidence that sympathetic nerve fibres form hypolemmal nerve endings on some striated muscle fibres."

2. The innervation of red and white fibres. As pointed out above, there has been evidence for some years indicating that the intrafusal striated fibres of the muscle spindles have an innervation independent of the somatic nerve, Sherrington (127) having shown that these muscle fibres did not degenerate after section of the peripheral nerve to the muscle, and Morpurgo (95) that they did not hypertrophy like other muscle fibres as the result of exercise. The recent morphological studies, especially those of Kulchitsky, point to the innervation of these intrafusal fibres as sympathetic in origin. Thus we have a muscle fibre of the red plasmic type innerved by a sympathetic nerve. In regard to other red muscles there are the early observations of Tcheriew (135) who found his "terminations en grappe" on those fibres which were "finer, less deeply stained and showing more accentuated longitudinal striations"; and also the work of Perroncito (105) who found such terminations exclusively on fine fibres, rich in sarcoplasm and with marked longitudinal striation. These descriptions coincide

precisely with the descriptions of red muscle, and draw attention to Kulchitsky's figures which show sympathetic innervation in the fine fibres only (see fig. 2). All these observations indicate that in the reptile the sympathetic terminations are on red muscle fibres. In the bird, however, Hunter and Latham (69) found no such clear distinction, but observed peculiar branchings of the sympathetic nerves,

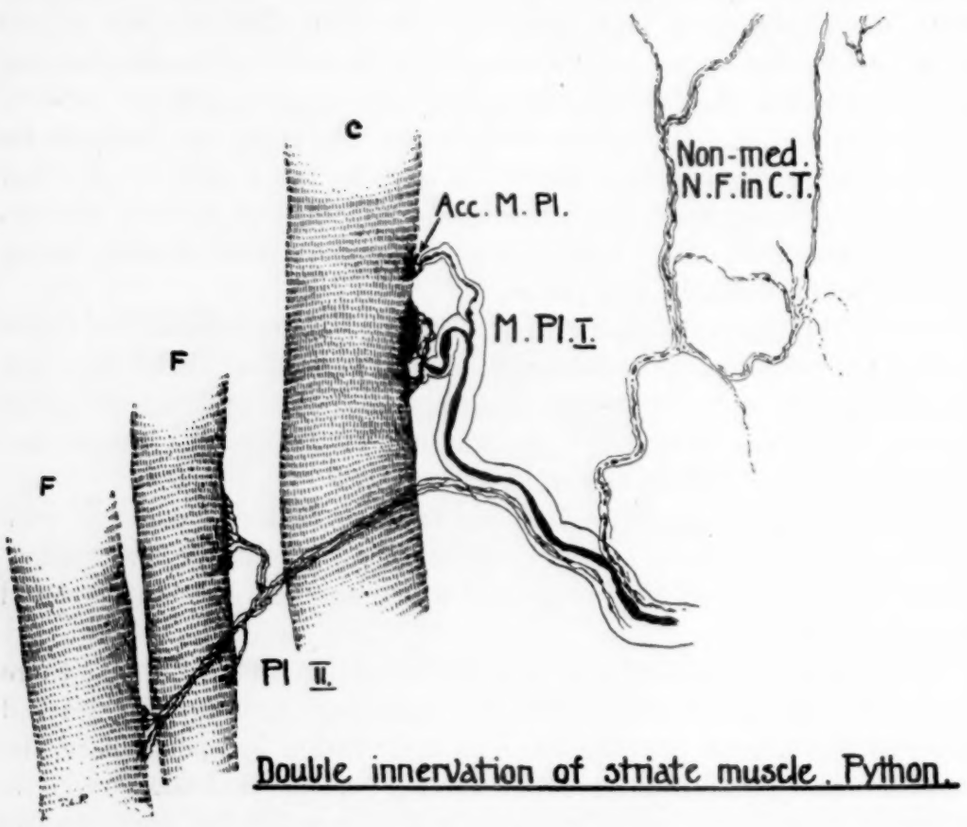

Fig. 2. Nerve endings in snake muscle (after Kulchitsky (77)). Two fine fibres, *F*, are shown with their sympathetic endings, and a coarse fibre, *C*, with its motor end-plate and nearby an ultraterminal ending (called "accessory motor plate" in this figure). The irregular plexus-like course of the sympathetic nerve is well shown.

"like maiden-hair fern," going to certain groups of muscle fibres, which were not obviously of the red variety nor different from those supplied by the somatic nerves. They also found that the sympathetic endings in goat muscle were different from those in man.

A paper by Hunter and Murray (70), not yet published, deals with the question—"Are the movement and fixing-muscles of invertebrates

separately represented as muscle-fibres of the skeletal muscles of vertebrates?" It is just this question that needs an answer, and the appearance of this paper is awaited with interest. At present the evidence indicates that fish, amphibians and reptiles may have two kinds of muscle fibres with different innervations. In birds and mammals, however, much evidence points to the lack of such a distinction; the red and white muscle fibres cannot at present be recognized as distinct and constant anatomical structures.

To add to the difficulties of applying this simple schematization of tonic innervation to mammalian muscle we have the careful work of Hay (60). He finds that in rabbit muscles there are always present both red and white fibres, usually more red than white. For experimental and microscopical investigation he used the semi-membranosus as an example of pale muscle and the semi-tendinosus and the soleus as examples of red muscle. His physiological experiments (quoted above) showed that these two differed characteristically in their contractions. Anatomical studies showed that the nerve fibres to the red soleus averaged smaller than those to the white semi-membranosus, but in both muscles similar motor end-plates were found (these are well illustrated in his plates VII, VIII, IX and X). Moreover the nerve trunk to a red muscle was always smaller than that to a pale muscle, and although it was made up of finer nerve fibres than the nerve to the pale muscle, bulk for bulk of muscle, the red muscle and the pale muscle were found to have a similar number of myelinated fibres in their respective nerve trunks. They also have a similar number of afferent fibres (61). These observations and those of Langley which show that there are more non-myelinated fibres in a nerve trunk to the skin than in a nerve trunk to a muscle, certainly make us pause in our acceptance of the theory that red muscle is innervated by sympathetic nerves and pale muscle by somatic. In addition Roberts' (118) degeneration experiments, following the cutting of the nerves to the soleus and gastrocnemius, show that both in degeneration-time and amount of wasting the red and the white muscles are approximately the same, if anything the red muscle shows more atrophy than the white. There is no evidence that atrophy follows cutting of the sympathetic nerves to a muscle. These facts would indicate that a majority of red muscle fibres in mammals must be somatically innervated.

From all this evidence it appears that *in mammals red and white muscles differ somewhat both in anatomy and physiology, but the distri-*

bution is variable and white fibres may perhaps turn red. The type of innervation gives no clear and consistent anatomical basis for distinguishing between red and white fibres. There appears to be, however, a clearer distinction in reptiles.

3. The question as to whether a single muscle fibre may have more than one kind of motor innervation. Kulchitsky, Hunter and Latham all believe that a single muscle fibre may have either a somatic motor or a sympathetic motor innervation, but *never both.* Dart (34) on the other hand, working with Kulchitsky's material finds a double innervation, that is, both types of nerve ending on every fibre. He refers to figures 1, 4, 8, and 12 which are entirely unconvincing, in fact 8 and 12 have nothing to do with the subject being pictures of muscle spindles. Boeke (16) also believes in the double innervation theory. His beautiful preparations show motor end-plates with their myelinated nerves, and "accessory" endings with non-myelinated nerve fibres, on the same muscle fibre. His identification of sympathetic fibres does not rest solely on the absence of myelin; he points out that the rare ultraterminal ending, and a common grape-like end-organ, both arise from somatic fibres. Although the fibres are non-medullated they are coarse, and can be easily distinguished from the fine sympathetic fibres which arise from irregular plexuses; also the very fine end-organs of the sympathetic nerves are unlike the coarse, though grape-like somatic endings. New investigations are needed to settle these disputed points, especially careful comparative anatomical work, to show whether or not lower vertebrates differ from mammals in the innervation of their muscle fibres.

4. The question of the contractility of sarcoplasm. The theory that sarcoplasm is an actively contractile substance, innervated directly, does not seem to be particularly substantiated by any anatomical facts. The nerve endings in muscle fibres have been especially well described by Boeke (12), (15). As far as one can determine from purely histological evidence these endings seem to connect directly with the contractile substance without the intermediation of sarcoplasm. Lieberkuhn's (91) observations on stentor, in which he saw slow contraction without the participation of the fibrils, can hardly be applied to highly developed mammalian muscle. Physiological evidence of sarcoplasmatic contraction is lacking, but it has been used by some as an argument to explain the mechanism of double innervation. This is, of course, contrary to the generally accepted idea that the function of sarcoplasm is metabolic (41)—a local food supply for the active

sarcostyles (132). Nevertheless the surmise of Botazzi (21) has had a considerable vogue. From observations on the batrachian heart he believed that "the sarcoplasm, in its tonic contraction, supports the anisotropic substance in its quicker contraction." Physiologists and neurologists from deBoer and Langelaan to Regelsberger (114), Stefani (133), Hunt (63) and Foix (42),—to quote a few of the more recent ones—have invoked this theory to explain tonic phenomena. That it has gained such wide credence is remarkable—perhaps it has happened merely through quotation, requotation and even misquotation.[5] *One cannot see that there ever has been any proof that the sarcoplasm in a muscle fibre is in fact contractile.* It is purely an hypothesis.

II. The question of a sympathetic influence on muscle tone. Since the early speculations of Mosso (96) concerning sympathetic innervation of striated muscle a great deal has been written on the subject. DeBoer's monograph (18) in 1913 first brought the question to the experimental test. He observed that cutting the rami communicantes of the abdominal sympathetic in frogs caused a loss of tone in the ipsilateral leg muscles. He also claimed that extirpating the abdominal sympathetic chain on one side in cats caused ipsilateral atony with exaggeration of the tendon reflexes. The loss of tone showed in the affected hind leg by its diminished resistance to passive flexion and by the fact that when the cat was held up by the skin of its neck, the affected limb hung lower than the contralateral one and showed greater extension at the joints. The animal's tail was observed to hang towards the unaffected side. There was also a great deal of evidence brought forward concerning the single twitch of tonic and atonic frog muscle, veratrine contracture, rigor mortis and the like. His work was quickly followed by papers from other men who tried with varying success to corroborate, amplify and criticise his results.

These papers have been reviewed and discussed by several authors, notably Adrian (2), (4), Langley (85) and Spiegel (131). The latter in 1923 analyzes the literature at length and shows that the original work of deBoer (19), (20) has been corroborated only by Langelaan; it has been refuted by van Rijnberk (140), Cobb (25), Dusser de

[5] Roberts' statement that Sherrington believes postural tonus to be "sarcoplasmatic" in origin is unwarranted. Nowhere in Sherrington's writings is there an expression of opinion on this point, nor in fact has he ever accepted the theory of sympathetic control over the tonus of striated muscle, as has been stated by other authors (72), (114).

Barenne (36) and Takahashi (134). Furthermore Negrin y Lopez and von Brücke (97), Yas Kuno (78), Saleck and Weitbrecht (123) in repeating deBoer's experiments had inconstant results, and found, after cutting sympathetic nerves, at best a passing and unconvincing loss of tone in the muscles involved. Spiegel concludes that impulses coming over the sympathetic chain have nothing to do with contracture, and that no unassailable proof can be found for the assumption that any static innervation of skeletal muscle passes by way of the abdominal sympathetic chain. He modifies this statement, however, by adding that it can not be said that the continuous contraction of muscles, when contracting involuntarily, has nothing at all to do with the sympathetic chain. He believes that one must consider that the lasting influence of the nervous system on the metabolism of resting muscle is at least partly subserved by the sympathetic system.

Since 1923 new stimulus has been given to the discussion by the work of Hunter and Royle in Australia who were invited by the American College of Surgeons to deliver in New York the Murphy Orations of the year (66), (120). This trip ended sadly in Hunter's death from typhoid in London on December 10, 1924.

The new evidence brought forward by these men reopens the subject and deserves careful consideration. The *starting points* of their research are: *a*, Kulchitsky's anatomical studies, and *b*, Langelaan's theory that tonus may be divided into "plastic" and "contractile" elements. They *build their theory on:* 1, the presence in striated muscle of two types of fibre—red and white; 2, the existence of a double innervation—somatic to the white fibres and sympathetic to the red; and 3, the presence in muscle of spindles with somatic afferent nerves and sympathetic efferents. They produce *new evidence* from 1, experiments on normal, spinal and decerebrate goats; 2, experiments on birds,[6] and 3, operations on man. Their experimental work may be briefly summarized as follows: in two goats the spinal cord was cut and simultaneously, or after a few days, one abdominal sympathetic chain was extirpated. The leg on the side of the sympathectomy was flaccid whereas the other retained some tonus and more reflex activity. In another series of five goats the abdominal sympathetic trunk was

[6] A paper by Trendelenburg (138) describes cutting the dorsal roots of the nerves to the wing in pigeons, and indicates that the nerve supply to the wing of a bird is quite different from that to the fore-limb of a mammal. Until this has been thoroughly studied it would seem wise not to apply the results of experiments on birds to explain motor phenomena of mammals' arms.

removed on one side. Four of these animals were allowed to recover and were observed for a period of from 14 to 73 days. For the first few days after the operation the animals limped slightly, after this had passed off there remained only a slight lack of resistance to passive flexion and some tremor in the affected hind leg. These animals were then decerebrated. Extensor rigidity appeared as usual in all four limbs, but when the animal was rolled onto its back the hind leg on which sympathectomy had been performed soon lost its extended posture and fell (under the influence of gravity) to a position of abduction and flexion. This leg was examined for the lengthening and shortening reactions of decerebrate rigidity, and they were found to be absent. Myographic records (66) were taken of the knee jerks of both hind legs, and while the normal limb showed a curve with prolonged relaxation period, the operated side had a shorter knee jerk, the curve dropping quickly to the base line. Other experiments on goats have been performed but data on the actual experiments are given only for those mentioned above, and for two others in which the findings seem of less importance (119).

Of even more interest are the experiments performed by Hunter on birds (65), (68). He operated on domestic fowl and on sea-gulls, cutting the sympathetic trunk just above the first thoracic ganglion thus removing the sympathetic supply to the whole wing. (The bird's brachial plexus and sympathetic connections are not like those of mammals, see Hunter (65) fig. 7.) After this operation the ipsilateral wing is somewhat drooped and after passive movement shows a tendency to return only slowly to its former position. Fatigue greatly increases this effect. To illustrate this sea-gulls were flown on a light line for 100 metres; at the end of this period they showed marked dyspnea, the abduction and drooping of the wing became very marked, and the bird made ineffectual attempts to place it in the usual position. Even without the effects of fatigue birds thus operated on are unable to maintain the maximum position of abduction and flexion against the action of gravity.

In another series of birds the posterior nerve roots of the four lower cervical segments were cut. After this operation, when the wing was stretched outwards and released, it dropped to the side of the bird instead of taking up the usual flexed attitude. If the wing were passively flexed it would remain in its new position. The reactions of these de-afferented wings are used by Hunter as illustrations of shortening and lengthening reactions.

Hunter's theory of muscle tone, in brief, is that it consists of two elements "*plastic tonus*" and "*contractile tonus.*" The former is recognized by the presence of the lengthening and shortening reactions; it acts on all muscles and *maintains a posture* once the posture is assumed; its effector organ is the slender muscle fibre, which is innervated only by sympathetic nerves. "Contractile tonus," on the other hand, acts differentially on various muscles, *imposes positions* and changes postures; it works through the somatically innervated white muscle fibres. Thus "the slender (red)[7] fibres innervated by the sympathetic, non-medullated nerves are not contractile (although their length is passively altered by the activity of the contractile fibres alongside them): their function is to establish plastic tone, to support and maintain the more active and easily exhausted contractile fibres and relieve them of much of the work of support that would otherwise induce the rapid onset of fatigue" (65).

It is not in the recent and frequently fragmentary pieces of work that the important criticism of Hunter's theory is to be found. Some of the investigators support the theory, but more dispute it. Most of them do not exactly repeat Hunter's experiments and so their results cannot be used as evidence to criticize directly his results. All, however, are useful, for the accumulation of various data alone can settle the question. It is from the papers of Hunter and Royle themselves that doubts arise as to the soundness of the theory. There seem to be four weak links in the chain of evidence. Two of these at least are essential to the theory.

In the first place there is the question of *red and white muscle.* In the beginning of Hunter's second lecture (65) is the statement that the "striated muscles of vertebrates consist of two kinds of fibres (in addition to the muscle spindles) and that the difference of their innervation is as distinct as the contrast in their anatomical features, one group being controlled by the spinal cord through medullated somatic nerves, the other by the central nervous system through the intermediation of the sympathetic system." As pointed out above this may be true of fishes, amphibians and reptiles; but there is much evidence which indicates that it is not true of mammalian muscle.

Another supposition on which Hunter's theory is based is that there

[7] Prof. Elliot Smith is a personal communication says—"In his published statements Hunter deliberately refrained from identifying the slender and coarse fibres with red and white muscle. For this suggestion about red and white muscle in the lectures I am responsible."

are *two kinds of tonus—contractile and plastic.* This he accepts on the grounds of Langelaan's papers (81), (82), for there is no direct evidence brought forward in Hunter's and Royle's own work that two such factors exist in tonus. They merely point out that their experimental facts are explainable on this theory and that only partial loss follows cutting one set of nerves. A careful perusal of Langelaan's papers fails to convince one that he has proved anything at all about the existence of two kinds of tone. He deals either with atonic muscle or with active proprioceptive reflexes set up by applying variable loads to the muscle. He finds plasticity in an atonic, denervated and devascularized muscle, and defines it as "a slow yielding to continuous stress." In other places (81) he seems to consider plasticity as the malleability or compressibility of muscle, something that one might measure with the external pressure of rubber cylinders. This is obviously a very different conception of plastic tone from Hunter's (66) who quotes Sherrington, and uses the term in the sense of a holding-mechanism, i.e., the ability of muscle to take up various new lengths without change of tension. Langelaan's general definition of tonus is good. He says "tonus designates the state of the muscle when continually subject to the influence of weak stimuli emanating from its own proprioceptors." *It is, however, unproven and improbable that there is any qualitative difference between "plastic" tonus and "contractile" tonus.* In this connection Forbes (45) says: "The foundation furnished by Langelaan's experiments for the theory seems to me wholly inadequate, consisting apparently in nothing more substantial than the superficial difference between the brief twitch resulting from a single artificial stimulus and the more gradual contraction observed in the case of certain tendon reflexes. This difference in time relations may readily be explained in the manner indicated by Liddell and Sherrington as resulting from a gradual increase in the number of constituent fibres of the muscle becoming involved in the response, without recourse to any assumption of a dual function of the muscle fibres. Furthermore Langelaan in seeking support for his theory of tonus as distinct from contractions evoked by stimulating medullated nerves, seriously misconstrued certain statements and observations of Keith Lucas. He cites Lucas as having found three different kinds of *contractile* substance in skeletal muscle, whereas Lucas clearly stated that he found three *excitable* substances, one of which he identified as the nerve fibres within the body of the muscle—obviously not to be classed as a contractile substance. The contrac-

tion of the muscle was the same in Lucas' experiment, no matter what excitable substance was acted on by the stimulus."

The third point is perhaps less fundamental, but still hard to explain. It is the question of the *interval which must elapse after operation* before the disappearance of plastic tone. Both Hunter and Royle point out the necessity of waiting days or weeks after cutting the rami in order to obtain the full effects. This is just the opposite from what one would expect of a sympathetic nervous reaction, and it leads one to think of a chronic trophic or vascular change in the muscles as the explanation for the late changes in tonicity. In fact even the morphological changes described might be due to such causes rather than to a direct denervation atrophy of the muscle fibres. Bailey (8) says with some justice: "The operation and the theory on which it is based will stand or fall on the immediate reduction of plastic tone following the operation. . . . I say *immediate* reduction of plastic tone, since plastic tone is a reflex phenomenon. That the patient does or does not have better motor function six days or six weeks or six months later is entirely beside the point and must be explained by extraneous factors."

Lastly the most unsatisfactory part of all the work from deBoer to Hunter is the *lack of positive experiments*—all are negative, only showing loss of function—there are no satisfactory stimulation experiments. Langelaan (83) has recently published a paper purporting to show the results of stimulation of the rami communicantes in frogs, but the method of stimulation, in so small an animal, is unreliable, and the changes measured are so minute that they are meaningless. Moreover, no intelligible data is presented showing how many experiments were done, how many were positive, how many negative, and giving plainly the actual measurements. In discussing the state of the muscle he says that after cutting the rami communicantes the muscle is "flabby" but "less deformable"; and stimulation of the rami increases "plasticity" and augments "tissue-tension." The terms are incoherent.

If tonus is a sympathetic nervous phenomenon stimulation of the sympathetic nerve should cause some change in the state of the muscle, but neither electrical stimulation[8] (25), (22) nor adrenalin (54) have shown any change, unless the recent experiments of Orbeli (99), (100) may be taken to prove that the decrease of the fatigue effect following

[8] The question of pseudomotor contraction cannot be taken up here, as mentioned above (page 518) its relation to the question of tonus is still uncertain.

sympathetic stimulation is related to increased muscle tone. In these important experiments he never refers to "tonus" but shows that sympathetic stimulation reinforces the irritability and conductivity of skeletal muscle. Thus he agrees with Pavlow that the sympathetic innervation of skeletal muscle is "trophic," i.e., is concerned with metabolism, and only indirectly with actual contraction.

In *summarizing* the weak points in Hunter's theory, one can say that *until it can be proved, (a) that mammalian muscle contains two distinct forms of fibre, the slender and the coarse, each with a special innervation, and until (b) there is satisfactory proof of the existence of two kinds of muscle tone, plastic and contractile—the theory of Hunter cannot be accepted. Additional evidence is also desirable to explain the "interval" after operation. Nor will the evidence ever be satisfactory until some positive proof is obtained through stimulation experiments.*

Beside the evidence gained from experimental work there is the surgical experience of Royle and others in the treatment of spastic paralysis by cervical or lumbar "rami-section." Royle (120) published in December 1924 the histories and results of some of his cases; at that time he had done more than 75 operations. He reports diminution of plastic tonus following many of the operations. The late results of some of his operations, however, have not been so good (33) and in the hands of others (71), (7) the operation has been less successful. At this time it is impossible to draw any conclusion from the surgical results; too many factors are involved; anatomical control is impossible, and not enough data have accumulated.

Hunter's work was all too short, and it is to be hoped that his interesting observations on goats and birds will be repeated. As Walshe (143) suggested, goats may differ from the usual laboratory animals in their reflexes and reactions. Royle's (119) description of the two spinal goats especially points to this possibility.

During the last year work has been done on tonus from various points of view.

Kuntz and Kerper (80) removed the lumbar sympathetic trunk on one side in ten dogs and compared the tonus, the power of contraction and the resistance to fatigue of the normal and the affected hind leg. This was done immediately after operation in six dogs, and after a lapse of from 10 to 36 days in the other four. All the animals showed a decreased resistance to fatigue in the muscles of the limb from which sympathetic innervation had been removed; this was even present when the blood supply to both limbs was cut off. There was also

hypotonicity in the muscles of the operated limb; in those animals which were allowed to survive this gradually disappeared. These results confirm Hunter and Orbeli, but the passing off of the hypotonicity during the days following operation is a phenomenon opposite to that observed by Hunter and Royle.

Foix (43) in a paper largely concerned with the electromyographic aspects of contracture and the clinical abnormalities of tonus mentions (p. 3, and figs. 1 and 2) experiments on dogs which showed loss of tone in the ipsilateral hind leg after removal of one lumbar sympathetic chain. This hypotonus was less marked than that following section of the lumbar posterior nerve roots.

Kanavel, Pollock and Davis (71) operated on 16 cats and 12 human cases. In the cats the abdominal sympathetic chain was removed on one side and the cats then were allowed to survive from 14 to 63 days, when they were decerebrated by ligation of the common carotid and basilar arteries. No difference in quality or maintenance of tonus could be distinguished on the operated side as compared to the normal. Twelve operations on patients suffering from rigid limbs (as the result of paralysis agitans, cord lesions, cerebral vascular lesions or Little's disease) were performed; the type of operation varied, but in all the limb was denervated of its sympathetic supply. None showed any improvement. In discussing this report Doctors Ryerson and Lewin, orthopedic surgeons, gave more optimistic reports of results of Royle's operations. Carlson, however, felt that "every critical experiment undertaken by a critical physiologist is absolutely negative."

Newton (98) does not believe that the sympathetic system influences the tone of striated muscle. In a series of rabbits operated on by him no hypotony of the leg was observed following lumbar excision of the sympathetic chain on one side, nor was there any diminution of heat production in the muscles of that limb.[9]

Riesser and Simonson (116) showed by experiments on the frog's gastrocnemius that certain poisons affecting the central nervous system, like cocaine, increase the tonic after discharge and cause a con-

[9] Since writing this paper I have had the privilege to observe results of the experiments of Doctors Cannon, Forbes and O'Connor at the Harvard Medical School on 17 cats which have had the sympathetic nerve supply removed from one limb and which have been observed over periods of from 3 to 80 days. No positive changes of tonicity in the muscles of the affected limb have been found. In short this work though still in progress and unfinished corroborates in all essentials the negative results quoted above.

tracture. This is not affected by removal of the fore-brain, the basal ganglia, or the abdominal sympathetic, but extirpation of the mid-brain abolishes it.

Plaut (110) observed in a man with amputated arm two functions of muscle, i.e., the patient could at will either harden the muscle without shortening it, or he could contract and shorten it. In experiments with the tonometer he found that decerebrate rigidity in cats differed from spasticity in man. He also found that the metabolism in decerebrate rigidity was lower than the work done would lead one to expect.

Gayda (54) in an article on the influence of adrenalin on the tone of striated muscle, shows that in dogs without narcosis there is no increase in muscle tone following the administration of adrenalin. He therefore believes that tonus does not depend on sympathetic innervation. The article is short and gives many references.

In a long and rather speculative paper Lenaz (86) proposes that tonus may be defined as that dynamic power to balance weights which is revealed by the variable resistance of muscle. This is found in resting muscle as well as in contracting muscle. He believes it depends on the degree of hydration of sarcoplasm which reaches an equilibrium in relation to the weight to be lifted: the muscle then shortens by the action of the fibrillar apparatus.

The earlier works of Pieron (106), (107) on tonus were merely reviews of the subject, arguing in favor of Langelaan's hypothesis. During the last year he has published with Ozario de Almeida (102), (103) experiments to show that afferent impulses from the skin have a great effect on reflex muscle tonus. The experiments are not carefully enough described to make accurate criticism possible, but they appear to have been too crude to have any value in explaining normal tone. No discussion is given of Sherrington's early work (130) where it was proved that tone and muscular coördination are quite normal in limbs in which the cutaneous nerve supply has been completely severed.

Langelaan's last contribution (83) deals partly with the structure of muscle in relation to tonus. Anatomical studies of muscle structure are needed, and the histological work is interesting, but the method of reconstruction employed is inexact and presupposes that muscular structures have geometrical shapes.

Creatin metabolism. Many authors use part of the experimental evidence concerning creatin metabolism to support the view that there is a double innervation of muscle (35), (1). Langelaan even believes that creatin enters into the "red muscle cycle" of tonic contraction to

an extent comparable to lactic acid metabolism in pale muscles. If this were true one would expect to find more creatin in red muscle than in pale muscle, but this does not seem to be the case. In addition, Langley (85) points out that the experimental work really indicates that creatin metabolism is more dependent on somatic innervation than on sympathetic.

It is true that creatin plays a part in muscular metabolism. Cathcart (24) in his recent review states that muscular work leads to a definite, though perhaps limited, increase in the output of nitrogen—an increased metabolism of protein. Spiegel (131) agrees that one must accept as a fact the increase of creatin with muscular work. In relation to tonic contraction, however, he emphasizes the point that no conclusions can be drawn from the divergent results of chemical research, and that much of the work is valueless because it fails to take into account the impeded circulation in the muscles studied. He believes that for tonic contraction, as for phasic, carbohydrate metabolism is the important mechanism. Most of the work since Spiegel's review is open to this same criticism, and the conditions of many of the experiments are too complex to carry conviction. For example, Looney (92) found an increase of creatin in the blood of catatonic dementia praecox patients and believes that the amount of creatin in the blood may be considered as an index of the tonicity of the muscles of the individual. Reading his case histories, however, we find such descriptions as the following: cases 1 and 4, "extremities cold and cyanotic," case 10, "extremities cold and blanched." These irregularities in circulation are sufficient to explain marked changes in the blood chemistry. Moreover, the patients were abnormal in other ways: some had to be tube-fed; case 2 had "extremities maintained in flexed position"; case 9 had "no stiffness at time of specimen." Poncato (111) found an increase in creatin in the urine of hysterical patients with muscular contracture. Uyeno and Mitsuda (139), in experimental work on frogs, found that creatin was increased in a muscle to which the circulation was cut off, and they suggest that the amount of creatin varies with the rate of blood flow.

Recent experiments by Dusser de Barenne and Cohen-Tervaert (40) show that there is no change in the creatin content of muscle occasioned by decerebrate rigidity, but that when phasic innervation with rhythmical contraction of the muscle is superimposed on the decerebrate rigidity, there appears a distinct increase in the creatin. They discuss the work of Pekelharing and others and conclude that decerebrate

rigidity does not give *per se* an increase in the creatin content of muscles. A recent paper by Hammett (59) shows that creatin formation is not a process essentially confined to the muscle tissue; while Tiegs (137) offers a theory that creatin may act as a basic substance to neutralize the lactic acid formed during muscular contraction. All this brings one to the conclusion that as yet *no satisfactory evidence has been produced that creatin metabolism is in any special way associated with tonic muscular contraction.*

Respiratory exchange. The ability of muscle to maintain contraction without measurable metabolism is a phenomenon which has received much attention. Many invertebrates, especially molluscs, seem to be able to contract their muscles and accomplish considerable static work with a remarkably small respiratory exchange (104), (9), (32). In mammals also tonic contraction certainly causes less metabolism than active muscular movements. This has been recently proved by Dusser de Barenne and Burger (39) who, with an improved apparatus, showed that rigid decerebrate cats had an oxygen consumption from 10 to 25 per cent higher than they do after abolition of the rigidity. These observations supersede the former negative experiments of Roaf (117). Active muscular movement causes a greater respiratory exchange. The difference, then, between the metabolism of tonically contracting muscle and that of muscular movement is one of quantity rather than of quality. Spiegel (131) also finds that *the metabolism of tonic muscle is less than that of actively contracting muscles:* he believes that those observers who find no respiratory exchange during the tonic state are using faulty technique. Perhaps as Langley (84) suggests there are several forms of muscle tone involving chemical change at the onset only. Spiegel assumes that the presence of tetanic action-currents in a contracting muscle indicates an active process with greater metabolism than a contraction in which no action-currents can be recorded. He believes that the latter should be looked on as a separate type of contraction (131). This theory is probably not sound, for the presence or absence of recordable action-currents cannot be considered a fundamental physiological distinction.

Electrical phenomena. It was first shown by Dusser de Barenne (38) that the tonic contraction of decerebrate rigidity was accompanied by a stream of action currents making a rapid multiphasic electromyogram. These observations have since been amply corroborated. Adrian (3) and Forbes and Cattell (47) believe that all muscular contractions of central origin are accompanied by oscilla-

ting action currents, but that they may not be detected by the galvanometer, either because of faults in the recording apparatus, or because of lack of synchronism in the action-currents themselves. They explain this by saying that at times muscle fibres respond more nearly in phase with each other than at other times. When large groups of fibres are responding in phase a dominant rhythm will appear in the electromyogram, but when there is no such "volley firing" (109) the action currents may be so completely out of phase as to neutralize each other, at least in so far as recording changes of potential in a galvanometer is concerned. In other words, perfect synchronism causes a great increase in the recorded electrical response without any increase in mechanical efficiency. A good example of this is shown by Langelaan (83) in his electro-myogram of a proprioceptive reflex. Here a tonic muscle is at first shown giving no action-current, but as soon as a pull is given to this muscle the slightest increment of tension is followed by rhythmic vibration of the galvanometer string. Langelaan concludes "that a variation in the tension of the muscle provokes reflexly a short phase of contractile tonus." A simpler explanation, according to Fulton and Liddell (50), would seem to be that tonus was always present in the muscle, but that increased tension caused an immediate volley of afferent proprioceptive impulses to set up an increased tonic contraction in the muscle which registers a series of action-currents in the electromyogram. The more sudden and brief the proprioceptive stimulus the more in phase will be the action-currents, witness the difference between the electromyogram of a knee-jerk, of various types of clonus and of a slow stretch reflex. In the knee-jerk there is only one quick diphasic response, in clonus there may be one to seven action-currents for each contraction (26) whereas in the stretch reflex the electrical response is either absent, or slight and irregular, according to the steadiness of the pull (50). Langelaan evidently has this same idea in mind when he says "contractile tonus is a state of slight disordered contraction of muscle."

Forbes (46) expands his observations into an interesting theory to explain the economy of energy seen in all tonic contraction. He postulates a "mechanism of rotation" by which a group of fibres in a shortened state sends to a limited number of motor neurons the requisite proprioceptive impulses to establish reflex contraction. The muscle fibres thus excited are different ones from those already in contraction. Synaptic fatigue may release these muscle fibres before they become subject to fatigue, but meanwhile their contraction has reflexly evoked

that of a third group. Thus the fibre-groups take up the load in rotation and by this means attain an economy otherwise impossible. Such a rotating mechanism would have action-currents almost entirely *out of phase* and would therefore register little or nothing in the galvanometer (3). Thus may be explained the lack of easily recordable action-currents in tonic contraction without recourse to the complex view of some investigators (131), (124), (56), (64) who maintain that the tonus of muscle is a distinct type of contraction fundamentally different from ordinary contraction, and devoid of action-currents. It is probable that certain states of *contracture* may occur in striated muscle without the accompaniment of action-currents, but these may well be due to abnormal chemical and physical conditions within the muscle fibre, not to excitation by the nerve impulse.

As a corollary to this belief that striated muscle can contract in response to nerve impulses without showing action-currents, we find many neurologists accepting the theory that the tonic contraction of striated muscle may cause a slow displacement of the galvanometer string. This is compared with the slow action-current of smooth muscle (51) and used as evidence for the double and sympathetic innervation of striated muscle. Foix and Thevenard (44) show a curve of this sort in their figure 2. Golla (55), Salomonson (124) and others have also given this interpretation to steady deflections of the galvanometer string. Adrian (3) in his recent lecture on the interpretation of the electromyogram quotes Lewy as also supporting this view, but points out that Einthoven and Cobb have quite clearly demonstrated that such curves are in all probability artefacts and due to improper fixation of the electrodes. Spiegel also holds this view. That the use of needle electrodes does not necessarily obviate this difficulty has been frequently observed by all workers with physiological preparations of exposed muscle. Observations of the action-currents of smooth muscle (101), (115) show that their amplitude is much greater and their velocity much slower than the action-currents of striated muscle. This is additional evidence that we have no right to compare such reactions in smooth muscle with those in red or white striated muscle and use the analogy as proof for the duality of striated muscle.

A recent paper by Wachholder (142) shows that in resting skeletal muscles when not completely relaxed there may be found occasional quick action-currents at the very low frequency of 5 to 10 per second. This is an important observation that should be corroborated for it

seems to have interesting bearings on the questions of tonus and posture.

III. The tonus of skeletal muscle as a proprioceptive reflex. Recent papers by Liddell and Sherrington (87), (88), (89), describe the *myotatic reflex*. This throws a flood of light on the mechanism of muscle tone, for it gives to one who would construct a physiological explanation of tone the simple *element*—the material—with which to build. The idea that simple proprioceptive reflexes form the basis of postural tonus is not new (2). Hoffmann (62) considered that the tendon reflex was such an elementary reaction; but this idea was never altogether reconcilable with the facts. The myotatic reflex as described by Sherrington (129), however, seems satisfactorily to represent the basic factor in the elicitation by gravity of the static geotropic reflexes of standing, and indeed of all postural tone. Briefly summarized, the discovery of Liddell and Sherrington is that even a gradual stretch applied to a muscle evokes contraction in it. This reflex is purely proprioceptive and is closely graded by the degree of stretch, which may be very small. For example, extending the muscle's resting length by less than one per cent produces a reflex contraction of even 2,000 grams tension. All muscles are not equally sensitive to stretch; the "antigravity" muscles give especially good myotatic reflexes; some flexor muscles apparently react to stretch only by inhibiting their antagonists; in most muscles there are two kinds of receptors, those which provoke reflex contraction (excitable by stretch), and others which provoke reflex inhibition.

Two other important characteristics of the myotatic reflex are its *persistence* of contraction and its intensely *local* nature. "Within limits, so long as the stretch increases, the reflex continues to increase. When augmentation of the stretch ceases, augmentation of the reflex contraction also ceases, and the reflex usually declines, merging into a long-lasting plateau-like contraction, which is maintained by the stretched-posture, consequent from the precurrent stretch-movement" (87). This maintained contraction *is* tonus; it is a proprioceptive reflex in response to the stretch of the muscle, whether that stretch is kinetic or static (87, fig. 2). "The passive stretched-posture acts *per se* as a stimulus," moreover, not only the particular muscle stretched but the portion of it stretched, reacts in this way. Thus the reflex is *local, graded* and *sustained,* and explains beautifully, both such exaggerated phenomena as the "standing reflex" of decerebrate rigidity,

and all the multitudinous changes of tone that follow and make possible our phasic movements. Truly, as Ramsay Hunt (64) expresses it, "Tone follows movement like a shadow"—in fact tone and movement coalesce and often cannot be separated, for they are built of similar material—reflex action—though they may differ in time-relation and source of stimulation. For example, in stepping the limb is moved by an interplay of flexion and extension, made effective by reciprocal inhibition, but this same inhibition not only prevents the antagonistic muscle from contracting, but prevents it from reacting in response to its own passive stretch. Only when the quick, phasic movement passes are the myotatic reflexes allowed to awake from their inhibition and delicately impose on the new position a more or less sustained posture (87), (90).

Viets (141) has shown conclusively that the essential elements of tonus are present in spinal cord reflexes. A flaccid knee-jerk may be made spastic by stimulation of the contralateral peroneal nerve, that is, by combining it with a crossed-extension reflex (see fig. 3). These effects are certainly regulated and sustained by bringing in higher integrations, but the elements of tonus and posture are present in the simple spinal reflex (75). The knee-jerk may be looked on as a "fractional form" (87) of the myotatic reflex; a form elicited by a short, sharp stimulus. Clonus is another fractional form, arising from pathological hypersensitivity of the reflex arc. The myotatic reflex, on the other hand, is a response to a gradual stretch, it is a graded physiological reaction. Thus Viets' demonstration of clonus appearing in the decerebrate cat when knee-jerks have raised the tonus of the muscle, and Cobb's (26) electromyographic record of clonus going over into muscular spasm (see fig. 4) both supply evidence from the pathological sphere, that knee-jerk, clonus, tonus and muscular spasm are built of similar material. The electrical phenomena (vide supra) in muscles during these various states corroborate this view and show that all these reactions in skeletal muscle are tetanic in character (28).

From all this evidence it can be seen that static and kinetic movements can be explained on the basis of one neuromuscular mechanism. "Tonic" and "phasic" reflexes, though unlike, are separated by no fundamental difference in physiological mechanism. Integration at various levels of the central nervous system, of course, complicates the picture; the reflex centre—be it low in the cord or high in the dienaphalon—provides the mechanism for sustained action. In general these centres follow the rule of encephalization, the more cephalad the

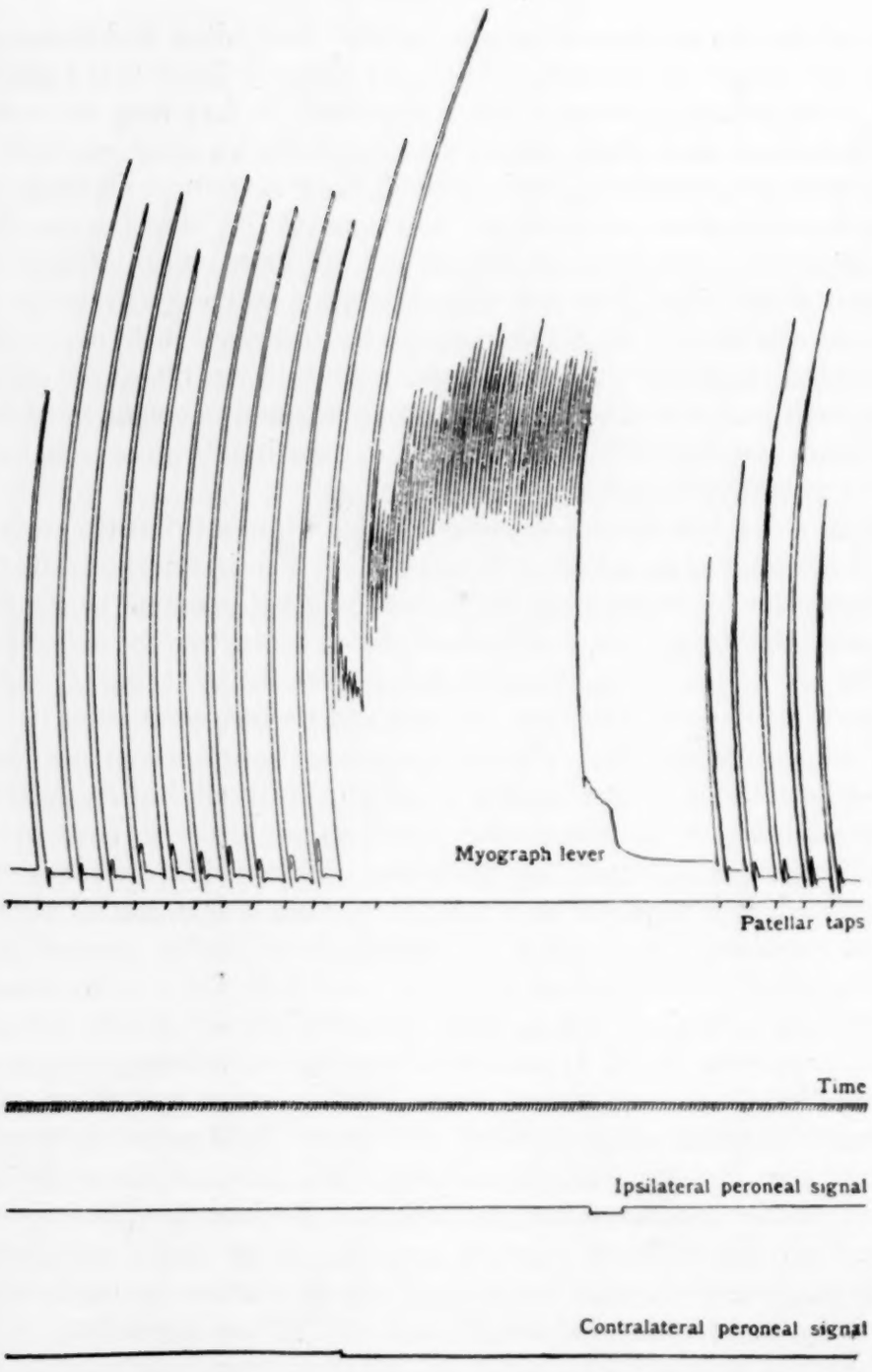

Fig. 3. Knee jerks in spinal cat augmented to clonus by contralateral peroneal stimulation (after Viets (141)). The patellar taps shown on the upper signal line cause knee jerk responses as long as the muscle is atonic, but when the contralateral peroneal stimulation (lower signal) has increased the muscle tone, two taps are sufficient to cause clonus. Ipsilateral stimulation causes inhibition of the clonus.

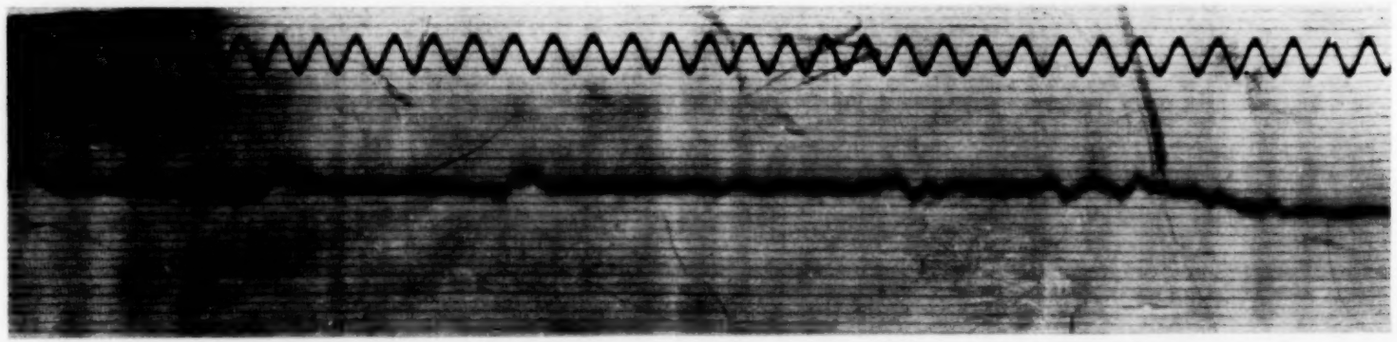

Fig. 4. Patella clonus developing into spasm; the electromyogram shows the transition from periodic to tetanic waves. Time is marked with a tuning-fork at the rate of 50 per second (after Cobb (26)).

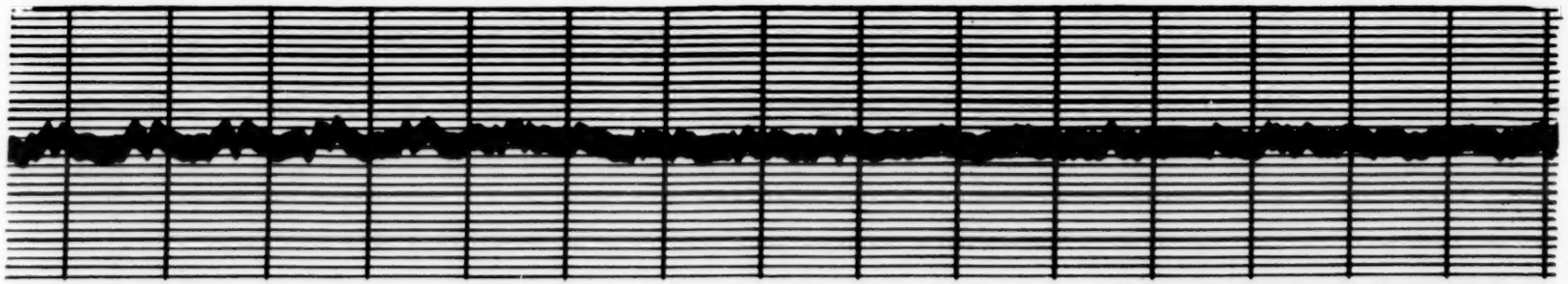

Fig. 5. Case of paralysis agitans. The first five periods of $\frac{1}{5}$ second each (marked by the vertical lines) show the typical action-currents of Parkinsonian tremor. The tremor then ceases and there remains only the fine frequent action currents which are probably due to the rigidity (after Cobb (27)).

centre the more sustained or tonic is its activity. This holds in a general way for all "tonus centres" from the cord to the basal ganglia. Above that is superimposed the cortex which produces for the most part isolated movements, supported of course by tonic reflexes.

Such tonus and posture as is found in spinal preparations is simple. We might consider the crossed-extension reflex as the most primitive example; here kinetic activity is followed by static activity; the after discharge is practically identical with tonus and electrically it gives action-currents similar to decerebrate rigidity (47). Thus for the crossed-extension reflex there is a more complex central mechanism than for such reflexes as the ipsilateral flexion reflex (48), and the spinal mechanism which subserves this rearrangement and maintenance of impulses can be looked upon as the lowest "tonus centre." Proceeding upward in the neuraxis we encounter in the upper cervical cord and in the hind-brain mechanisms which strongly effect tone, the afferent impulses coming from the proprioceptors of the neck muscles. Just above this the vestibular apparatus contributes its dominating influence, clearly described by Magnus (93) and de Kleijn (74). There may also be a pontile centre (65), and the mid-brain (containing the red nucleus and substantia nigra which are so closely connected with the basal ganglia of the fore-brain) plays an important part. Space does not permit a discussion of this complex problem, but various diagrams have been published which attempt to explain the interplay of these tonus centres and their effects on the muscle through the final common path of the peripheral neurone (145), (29), (65).

To summarize: the work of such objective observers as Sherrington and Magnus with their numerous collaborators, has given an explanation of the tonus of striated muscle which needs support from no dualistic theory of muscular function or innervation. It is known that smooth-muscle has tone even when isolated from the nervous system. Striated muscle shows no such intrinsic tonus; all its contractions, sustained or otherwise, are dependent on reflex action. For tone by far the most important afferent paths are those arising in proprioceptive organs—muscular or vestibular. The simplest reflex result of these afferents in the muscle is the myotatic reflex and from it may be built up by central nervous integration all the phenomena of postural contraction, excepting those that have their origin in the vestibular apparatus. It is possible that this is only one part of tonus—the part called "contractile tone" by the protagonists of the dual theory. More work is needed to corroborate and explain their findings. At

present the better evidence points to the simpler explanation: *that tonus is a beautifully graded series of proprioceptive reflexes, continuously and unconsciously playing its part in our every motor act. By its remarkable specificity it moulds our individual muscles; by its universality it controls our postures.*

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THE CHEMICAL REGULATION OF RESPIRATION

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The survey of the chemical regulation of respiration is a precarious adventure. One need only refer to the numerous reviews and monographs (1 to 12 inclusive) on this and related subjects to realize the enormous amount of data which is accumulating. Innumerable experiments under similar conditions repeated by competent investigators give certainty to findings, but the explanation of the results still awaits final proof.

The subject of respiratory control has probably given rise to as many theories and as much argument as any single phase of physiology. This is not at all surprising when we consider the scope of the subject. On the contrary, if careful stock is taken of what is known and of what should be known, it is surprising that the theories meet the facts as well as they do.

The chemical regulation of respiration implies a central structural mechanism of control, the respiratory center. But what accurate information is available on the center? Is it automatic or does it function as a reflex center (12a)? To what extent is its activity controlled by so-called nervous influences; and to what extent is it controlled by chemical agencies? What are the chemical agencies at work? Is it the hydrogen ion, carbon dioxide as such, or oxygen; or are there unrecognized elements or compounds regulating respiration? Is it the concentration of respiratory hormones in the arterial blood which stimulates, or is the metabolism of the respiratory center itself an important factor to be considered? In that connection, is the transport of acid to or away from the center the controlling element? What is the significance of the acidity (C_H) of the blood? What is the relation of the acidity of the blood to the acidity of the interior of the cells of the respiratory center? In what form is carbonic acid carried in the blood, and how across the cell membrane? What is the significance of the permeability of the cell membrane in the exchange of acid and base between the cell and its environment? What is the significance of the

buffer capacity of the cell as well as of the blood? These are some of the questions which need elucidation.

It must be evident that much of the experimentation on respiratory control has been planned to test empirical findings, and very little if any has been directed towards a specific knowledge of the behavior of nerve tissue. To be sure, work must be continued in every possible direction, but the final solution will come only through basic experiments bearing on the nature of the initiation and conduction of the nerve impulse in nerve and nerve centers. It is only then that the influence of various chemical factors can be understood.

Be that as it may, the subject must be critically reviewed with the data at hand. The complexity of the topic must serve as my excuse for errors committed in the criticism of theories of fellow physiologists, and for errors in a newer theory which I propose to elaborate.

THE HYDROGEN ION CONCENTRATION OF THE ARTERIAL BLOOD AS A REGULATOR OF RESPIRATION. It is generally believed that the hydrogen ions of the arterial blood stimulate the respiratory center thereby controlling its activity, and that pulmonary ventilation is so regulated as to maintain a constant hydrogen ion concentration of the arterial blood. This view rests primarily upon the work of Haldane and his associates, Winterstein and Hasselbalch. For Haldane and Priestley (13) in 1905 had shown the extreme sensitivity of the respiratory center to changes in alveolar carbon dioxide tension: an increase of only 0.2 per cent sufficing to double the minute volume; and a similar decrease sufficing to stop respiration completely. This exceedingly fine response to carbon dioxide was confirmed in 1914 (14). Winterstein (15) in the meantime had perfused young rabbits with acid solutions, and concluded that neither the lack of oxygen nor carbon dioxide as such, but rather, the hydrogen ion concentration of the blood served as the chemical stimulus to respiration. Immediately following this came the support of Hasselbalch (16), who showed that the resting alveolar carbon dioxide could be varied by diet, the hydrogen ion concentration of the blood remaining constant. Accepting the views of Winterstein and Hasselbalch, Haldane and his associates (17) modified their views on the regulative action of arterial carbon dioxide, and gave their results in terms of arterial hydrogen ion concentration:

> A rise of 0.2 per cent or 1.5 mm. in the CO_2 pressure of the alveolar air and arterial blood causes an increase of about 100 per cent in the resting alveolar ventilation, and from figure 54 it will be seen that this corresponds to a difference of 0.012 in pH. This difference, large as its physiological effect is, cannot be de-

tected with certainty by the electrometric method, or by indicators, and is quite undetectable by the shifting of the dissociation curve of oxy-haemoglobin. Nevertheless a twentieth of this difference would produce an easily measurable effect on the breathing or alveolar CO_2 pressure. The astounding delicacy of the regulation of blood reaction is thus evident. No existing physical or chemical method of discriminating differences in reaction approaches in delicacy the physiological reaction. Unfortunately, however, the quantitative significance of our calculation has not yet been appreciated. The blood within the living body is still treated as if its reaction were not only variable, during rest, as it is, but capable of showing the variations by the existing very rough chemical and physical reactions. One might as well try to cut delicate histological sections with a blunt carving knife, as try to demonstrate ordinary very minute changes in blood reaction by the existing physical and chemical methods.

These views of Haldane, Hasselbalch and Winterstein are accepted today despite the fact that a survey of the literature reveals innumerable exceptions to the correspondence between pulmonary ventilation and the hydrogen ion concentration of the arterial blood.

Lacquer and Verzar (18) employing carbonic and mineral acids as respiratory stimulants were the first to contradict the conclusions of Winterstein. Finding carbonic acid more effective for a given hydrogen ion concentration of the perfusate than the mineral acids, they believed that carbonic acid exerted specific effects independent of acidity. Hooker, Wilson and Connet (19), in well-controlled experiments, materially strengthened the views of Lacquer and Verzar. Scott (20) next administered carbon dioxide to decerebrate cats before and after intravenous injection of sodium bicarbonate, and found that carbon dioxide stimulated with equal effectiveness regardless of the hydrogen ion concentration of the blood. And, finally, Dale and Evans (21) arrived at equally striking results on the chemical regulation of circulation by varying the hydrogen ion concentration of the blood in different ways. A decrease in the hydrogen ion concentration of the arterial blood produced by over-ventilation was accompanied by a large drop in mean blood pressure which was entirely missing (or the blood pressure was even increased) when a similar change in the hydrogen ion concentration occurred as a result of the injection of sodium bicarbonate. This group of experiments should have discounted the regulative action of the hydrogen ion concentration of the arterial blood more than it did, especially since Jacobs (22), (23) had suggested an explanation of the action of carbon dioxide.

Reviewing a different type of data—the exceptions continue to be the rule. Haldane, Kellas and Kennaway (24) find hyperpnea and alkalemia in man at high altitudes. Haggard and Henderson (25)

simultaneously report the same results. Fraser, Ross and Dreyer (26) report hyperpnea and alkalemia in cardiac disturbances, and Barr and Peters (27) similar conditions in anemia. Bald (28) studied the effects of hemorrhage and subsequent injection of gum-saline solution, and found that hemorrhage was associated with increased ventilation and increased alkalinity of the arterial blood, and that injection was followed by decreased ventilation and increased hydrogen ion concentration. Anoxemia, hyperpnea and alkalemia seem to identify this group of experiments.

Haggard (29) concluded that the hyperpnea produced by hot baths is associated with alkalemia. The direct observations of Bazett and Haldane (30) agree, and Koehler (31) finds alkalemia in hyperpnea of fever. Hawkins (32) finds great individual and also wide daily variations in the arterial hydrogen ion concentration of the guinea pig and rabbit as compared with the rat, and notes that the guinea pigs and rabbits have a much less stable body temperature.

Cullen and Robinson (33) showed that the pH variations in normal individuals under normal conditions were considerably greater than was believed—from pH 7.28 to 7.41.

Mellanby (34) studied the effects of lack of oxygen, the administration of carbon dioxide, and intravenous injection of sodium carbonate and lactic acid, and found that carbon dioxide and oxygen lack produced marked increases in respiration with very little change in the reaction of the blood, while lactic acid and sodium carbonate produced great changes in the reaction of the blood and small effects on ventilation.

Capp, Foote and I (35) showed that acceleration of unchanged blood through the respiratory center was sufficient to transform hyperpnea to apnea. I stated later (36) that the "Parallelism between pulmonary ventilation and arterial pH is an accidental occurrence prevailing only under special conditions;" and I offered a theory of control based on changes in acidity of the respiratory center itself determined by its own acid metabolism and the transport of acid from the center, as opposed to the acidity of the arterial blood. This theory seemed to offer an explanation of the exceptions noted above, but later papers still support the regulation of respiration by the arterial hydrogen ion concentration. Endres (37) states that it is not the increased carbon dioxide as such, but rather its effect on the hydrogen ion concentration of the blood which acts as the respiratory stimulus, and thus explains the increase in arterial hydrogen ion concentration during sleep (38) as due to a lowered irritability of the respiratory center. Douglas and Priestley (39) studied

the effect of sugar ingestion on breathing, and found increased liberation of carbon dioxide. They state that

> On the cH theory of regulation of breathing the ventilation of the lungs should vary in proportion to the mass of CO_2 given off. Thus to account for the slight alteration in the alveolar CO_2 pressure shown in our experiments the cH of the arterial blood might actually have been a little higher than it was during the preliminary period in spite of the slight fall in the alveolar CO_2 pressure.

These recent papers are only a few of many which indicate how firmly the views of Haldane and Hasselbalch are anchored. Similar views are expressed in the report of the Haemoglobin Committee of the British Medical Research Council (40). In this thorough resume only casual reference is made to the acidity of the respiratory center. The importance of arterial hydrogen ion concentration is still stressed:

> A change in hydrogen ion concentration may be the primary cause of the alteration of the activity of the respiratory center. Under other circumstances the primary factor may be either shortage of oxygen or rise of temperature: both these conditions cause increased breathing and therefore will themselves tend to cause alkalemia. Here the change in the reaction of the blood is a secondary factor, but directly it is established its influence will obviously have seriously to be taken into account. The sensitiveness of the respiratory center to changes in hydrogen ion concentration is so great that an apparently trifling alteration of hydrogen ion concentration in the blood may result in great changes in breathing, and as the present methods of direct determination of the hydrogen ion concentration are often of insufficient delicacy to give us the accuracy of measurement that we require we are frequently forced to infer these changes by indirect methods involving measurement of the concentration of CO_2 in the arterial blood, and of the relative excretion of acids and bases in the urine.

And, to quote again in connection with increased respiration in diabetic coma:

> However, it should be pointed out that even if no measurable increase of hydrogen ion concentration is found in diabetic coma, it is possible that the change may be so slight as not to be capable of measurement by the relatively coarse methods at our disposal and it may yet be sufficient to produce the characteristic respiration of diabetic coma.

Means (41), in his recent review of dyspnea, offers the same explanation. Basing his conclusion on the work of Haldane and Priestley (13), he states that "Ordinarily carbon dioxide plays the major rôle in regulating the respiration and the rate of ventilation is adjusted to the carbon dioxide requirements. For purposes of oxygen absorption alone the ventilation is ordinarily in excess." He believes that "sufficient pul-

monary ventilation must be supplied to remove the carbon dioxide produced by metabolism, but at the same time its rate of removal must be so adjusted to its rate of addition to the blood that its tension will bear a nearly fixed ratio to the blood bicarbonate, this in order that the blood may be kept from more than slight alteration."

And using carbon dioxide diagrams of Haggard and Henderson he arrives at the following conclusion:

> As we study the blood in disease under certain circumstances we find carbon dioxide curves occupying positions lower than normal, as for example curve II in figure 8. If, however, we plot the arterial points for such curves, we often find as is shown in curve II, that there is little or no tendency toward change in hydrogen ion concentration. The arterial points tend to fall on a single diagonal or on diagonals in close proximity. This fact has led to the modification of the original Haldane-Priestley theory to the belief that what the respiratory center really reacts to and what in truth regulates lung ventilation is blood hydrogen ion concentration, not carbon dioxide tension. It is to preserve blood neutrality, not blood carbon dioxide tension, that the mechanism is designed. Of course with dissociation curves at a constant level carbon dioxide tension will remain constant as well as hydrogen ion concentration, but when the level is changed by disease or in other ways, as in curve II, it is found that it is the hydrogen ion concentration which the organism seeks to keep constant, not the carbon dioxide tension. This principle was first discussed by Henderson in 1909 and was later developed by Winterstein and Hasselbalch and accepted by Haldane.

Means states, "A still more modern theory is that the reactions of the tissues of the center itself is the true stimulus cf. R. Gesell." This newer theory has subsequently gained support in the findings of Barr (42) on the relation of arterial hydrogen ion concentration to pulmonary ventilation following muscular exercise. He finds no constant relationship between hydrogen ion concentration and breathing, also that the respiratory center following exercise is much less sensitive to changes in arterial hydrogen ion concentration than formerly proposed. He states

> It is, of course, possible that blood reaction does furnish the normal stimulus to breathing and that the lack of apparent relationship between reaction and respiration in our experiments and in those of others is due to changes in the sensitivity of the respiratory center under varying conditions. If this is true it is apparent that there must be many influences which modify or even obliterate the effect of the normal stimulus.

This is an important point. Even granting that acidity of the blood or of the center is the regulative stimulus, it would be exceedingly remarkable if the behavior of the center were not modified in other ways.

It has, of course, been frequently suggested that the response of the center to the hydrogen ion concentration is increased by a lack of oxygen. In fact every exception to the parallelism between pulmonary ventilation and arterial hydrogen ion concentration of the blood has been explained in some such way as this. Without such modification the theory of acid control would necessarily be abandoned and therewith the most effective respiratory stimulus. The abandonment of acidity as a stimulus apparently has seemed unwise.

But if acidity as the primary stimulus is not abandoned, the lack of parallelism between pulmonary ventilation and arterial hydrogen ion concentration must be satisfactorily explained. Granting that the acidity of the blood and tissues may vary in opposite directions, and substituting acidity of the respiratory center itself as the controlling factor for acidity of the blood, many of the difficulties of explaining pulmonary ventilation under a variety of conditions appear to vanish. It may even be profitable to ask whether acidity (C_H) of the blood as such is at all related to pulmonary ventilation (36).

It seems to be generally assumed, at least as far as acidity goes, that the blood and tissues are in perfect equilibrium; but no conception contradicts the function of circulation and respiration more than this does, for the exchanges between tissue and blood are based on diffusion gradients which are dependent on a lack of equilibrium, as exemplified in differences in tension or concentration of materials on two sides of a membrane. The function of circulation and respiration is nothing more than the maintenance of adequate diffusion gradients between the tissue cells and their environment, the tissue fluids of the body.

The simplest demonstration (43) of the lack of correspondence between acidity and acid effects is the stratification of two buffer mixtures of different concentrations, but of the same acidity. If two solutions of sodium bicarbonate—one containing 20 parts and the other 60 parts of sodium bicarbonate respectively—are saturated with carbon dioxide with the use of phenol red up to the same hydrogen ion concentration, carbonic acid and sodium bicarbonate will occur approximately in the ratios of 1/20 and 3/60. If these solutions which have the same color are now stratified a ring of colors develops at the interface, showing that the 1/20 solution is turning less alkaline and the 3/60 more alkaline—a striking demonstration of the possibilities in the living organism of lack of correspondence between acidity of the blood and acidity of the tissues! The acid effect of the 3/60 solution is explained by the relative rates of diffusion of carbonic acid and sodium bicar-

bonate, and the carbonic acid moving more rapidly than the sodium bicarbonate disturbs the ratios in the direction indicated by the colors. These effects must, of course, be accentuated in the living organism where the cell is separated from its environment by a semipermeable membrane, as it is generally believed that all cells offer a high resistance to the passage of metallic cations. In that event the acid effects of the environment on the interior of a cell may be independent of the acidity of the environment.

With this in mind Lashmet (44) studied the acid effects of bicarbonate buffer mixtures of approximately the same acidity on the hydrogen ion concentration of the red blood corpuscles, using the amount of oxygen combined with the hemoglobin as an index to the acidity of the interior of the cell. He suspended corpuscles in 1/20 and 3/60 solutions the osmotic pressures of which were adjusted with sodium chloride. Though keeping the 3/60 artificial plasma slightly more alkaline and equilibrating with a slightly higher oxygen tension the cells in the 1/20 plasma were found to take up more oxygen. The indications, therefore, are that the more alkaline solution exerted the more acid effect.

Miss Gayler (45) studied the acid effects of similar solutions of the same hydrogen ion concentration on vasodilatation of the pithed frog, employing the methods of Hooker. Accepting the view that peripherally acid produces vasodilatation, she found that the 3/60 solution exerted the stronger acid effect.

Perhaps the most convincing demonstration of the lack of parallelism between pulmonary ventilation and arterial hydrogen ion concentration is the actual record of what is happening in the living organism. With the aid of a manganese dioxide electrode placed directly in the circulating blood the hydrogen ion concentration of the arterial blood has been electrometrically recorded on smoked paper (46). A continuous picture of the hydrogen ion concentration changes along with respiration, blood pressure, and basal metabolism have yielded crucial data. Take, as an example, the effects of carbon dioxide and sodium bicarbonate. The administration of carbon dioxide increases the hydrogen ion concentration of both the arterial and venous blood but the arterial more than the venous, indicating that the tissues including the respiratory center have absorbed carbon dioxide and become more acid. This, of course, should explain the hyperpnea. On the other hand, injection of sodium bicarbonate produces virtually the same decrease in hydrogen ion concentration in the arterial and venous blood, indicating that very little sodium bicarbonate has left the blood stream. If true, the tissues

including the respiratory center were not alkalinized which would explain the usual absence of effects of sodium bicarbonate on respiration.

The continuous method of recording the hydrogen ion concentration of the circulating blood reveals the frequency of lack of parallelism between pulmonary ventilation and arterial hydrogen ion concentration. The administration of rarefied air elicits hyperpnea and alkalemia. Subsequent administration of room air elicits apnea as the arterial blood is turning acid. The greater the lack of oxygen the greater the pulmonary ventilation and alkalemia. And, subsequently, the higher the tension of oxygen, the more rapid and the greater the increase in arterial hydrogen ion concentration the more prolonged the apnea. Frequently increases in the hydrogen ion concentration amounting to 0.2 pH were accompanied by the development of apnea. In hemorrhage the results of Bald (28) were confirmed.

It is impossible to make an all inclusive statement covering the relation of acidity of the arterial blood to pulmonary ventilation. There is at present no evidence showing a causal relationship. The inverse relationship is more common than the direct. The reason for this will be further elaborated in other sections of this paper. It might only be indicated that an approximate constancy of the arterial hydrogen ion concentration is no proof that the hydrogen ions of the blood regulate respiration. The hydrogen ion concentration of the arterial blood may be the result of pulmonary ventilation rather than the cause. If acidity of the respiratory center itself is important, it is plausible that the diffusion gradients which govern acid-base equilibrium are adjusted to maintain a constant hydrogen ion concentration of the tissues, rather than of their environment, the blood. In that event, a lack of correspondence between pulmonary ventilation and acidity of the blood may find an explanation.

THE SPECIFIC EFFECT OF CARBON DIOXIDE INDEPENDENT OF ACIDITY. For many years the experiments of Winterstein (15) appeared to dispose of the possibility of a specific effect of carbon dioxide. The experiments of Lacquer and Verzar (18), contradicting Winterstein's conclusions, attracted very little attention, for about the same time the clean-cut results of Hasselbalch (16) appeared indicating an inverse relationship between pulmonary ventilation and alveolar carbon dioxide, and a direct relation to arterial hydrogen ion concentration. Only after the positive results of Hooker, Wilson and Connett (19), and of Scott (20) was the possibility of specificity reconsidered. Jacobs (22), (23), (47), (48) was at this time experimenting on unicellular organisms, and found that

an alkaline environment may exert acid effects on the interior of the cell. In a bicarbonate buffer mixture the requirement for such effects is a high carbon dioxide tension. The carbon dioxide diffuses into the cell and combines with water to form carbonic acid.

Later Dale and Evans (21) found that over-ventilation of the lungs produced the same fall in blood pressure after the injection of acid as before. Injection of sodium bicarbonate which lowered the hydrogen ion concentration of the blood and increased the HCO_3' ion, and injection of acid which increased the hydrogen ion without increasing the HCO_3' ion were both without effect. They concluded, therefore, that

> the results are in line with a number of observations which have appeared during recent years and which seem to indicate that free carbon dioxide as such, and apart from the increase of hydrogen ions produced by its addition to the blood has a specific stimulant action on the respiratory center; that the meaning of this specific effect of CO_2 as distinguished from acidity is by no means clear; that the fall of arterial pressure, which has been the subject of this investigation, is the result of acapnia in the original sense given to the term by Mosso, as accepted by Henderson in his earlier paper dealing with excessive ventilation and not "alkalosis" as more recent speculations have suggested.

Collip (49) found an increase in pulmonary ventilation on the injection of sodium bicarbonate comparable to the increased blood pressure described under similar conditions by Dale and Evans. He suggests a specific action of the HCO_3' ion.

The significance of these various results seems of such importance that I should like to recall an explanation which I offered on previous occasions (43), (36). Carbon dioxide in solution in water occurs in the different forms $CO_2 \rightleftarrows H_2CO_3 \rightleftarrows H \cdot HCO_3'$. Addition of sodium bicarbonate pushes the reaction to the left increasing the dissolved CO_2 and undissociated H_2CO_3 at the expense of the dissociated acid. Accepting Loeb's (50), (51) statement that weak undissociated acids diffuse freely through cell membranes, and Jacob's view that "the ions as such do not penetrate to any appreciable extent," it follows that the intravenous injection of sodium bicarbonate increases the freely diffusible forms of carbon dioxide in the blood at the expense of the poorly diffusible ions—thus increasing the acid effects of the blood on the interior of the cells, even though the total amount of dissolved carbon dioxide in its various forms is not increased and the hydrogen ion concentration of the blood is actually decreased. If the views of Loeb and Jacobs are correct, these effects alone should lead to the combination of acidosis

(tissue acidity) and alkalemia by virtue of the damming back of carbon dioxide in the tissues. The hyperpnea, the rise in blood pressure on the injection of sodium bicarbonate may thus be explained.

But this explanation neglects entirely the effect of the $H^{\cdot}$ and HCO_3' ions. Is this justifiable in the light of our present knowledge of ionic exchange (52)? We know, for example, that the hydrogen ions within the blood shift with extreme rapidity between the corpuscles and the plasma with every circuit of the blood, and we suspect that lactic acid also leaves the tissues in the dissociated form. At least, that is to be expected if the metallic cations do not shift. If so, there appears to be no reason why dissociated carbonic acid should not also diffuse, which suggested another stimulating effect of sodium bicarbonate. Arrhenius (53) showed that the addition of a salt to a solution of its acid accelerated the rate of diffusion of the acid from the solution. The addition of sodium chloride to hydrochloric acid, e.g., increases the concentration of the chloride ions and, therefore, their tendency to diffuse to a region of lower concentration. The chlorine ions cannot, of course, leave by themselves, and must take either sodium or hydrogen ions with them. The hydrogen ions being the more mobile are taken in larger amounts, and as a result acid diffuses more rapidly than before.

With the use of membranes Loeb (54) demonstrated the diffusion of acid against a positive gradient. This phenomenon is independent of a membrane and is easily demonstrated by the method of stratification of solutions described above. I prepared a solution of hydrochloric acid of a pH of 2 with the use of thymol blue as an indicator and divided this solution into two parts adding sodium chloride to one. The solutions were then stratified in a cylindrical separatory funnel, placing the denser solution below. A disturbance in the hydrogen ion equilibrium occurred at the boundary as rapidly as stratification could be accomplished with a display of colors indicating greater acidity above and lesser acidity below the original interface. With this method it is easy to demonstrate the diffusion of acid against very large positive gradients. Such a gross movement of ions involved in a visible demonstration of ionic exchange between acid and salt cannot help but emphasize the significance of ionic control of acid-base equilibrium between the cells and their environment where the surfaces are great and the effective distances of migration producing changes in acidity are small.

A physiological application of this phenomenon is perhaps found in the hyperpnea produced by the intravenous injection of strong solutions of sodium chloride. Such injection should theoretically lead to a migra-

tion of chlorine ions from the blood into the respiratory center, displacing, let us say, HCO_3' ions which leave the center for the blood. This exchange would be in favor of increased acidity of the center.

Applying these observations to another important salt and acid in the body, sodium bicarbonate and carbonic acid, the conditions are more complex. The intravenous injecton of sodium bicarbonate diminishes the hydrogen ion concentration of the blood, but in so doing it not only increases the amount of freely diffusible CO_2 and undissociated H_2CO_3, but adds a driving force which increases the migration of the remaining hydrogen ions. The addition of an alkaline salt must of necessity increase the diffusion of alkali as well as acid, which would tend to counteract the acid effect demonstrated in the case of sodium chloride and hydrochloric acid. On the other hand, if the tissue cells offer a differential barrier barring entrance to the metallic cations and admitting only acid, the acid effects in the interior of the cell would prevail. Theoretically, at least, the intravenous injection of sodium bicarbonate simply by increasing the concentration of CO_2, H_2CO_3, and HCO_3' ions in the blood prevents the diffusion outwards of the CO_2, H_2CO_3 and the HCO_3' ions to which the $H^{\cdot}$ ion is attached. The acid formed in the tissues should, therefore, accumulate. If this is true it is a principle of utmost importance in the physiology of respiration. I suggested this as a possible explanation of the hyperpnea following the injection of sodium bicarbonate.

These views on ionic exchange in the excretion of acid by the tissues into the blood have recently been supported by Collingwood (55). He bases his belief on the importance of ionic exchange in the exceedingly slow neutralization of carbon dioxide by an alkaline solution. He believes that the alkali liberated by the hemoglobin as the blood flows through the tissues would not combine with the carbon dioxide, and, therefore, this reaction would not facilitate the diffusion of carbon dioxide from the tissues into the blood. He suggests that the exchange of acid is largely one of ions. The mechanism of exchange which he proposes appears to differ from mine principally in that he allows the hydroxyl ion to enter the cell from without to replace the HCO_3' ion, which has left the cell, whereas I allow the HCO_3' ion to withdraw a hydrogen ion on leaving the interior of the cell. Both transfers undoubtedly occur and the results are, of course, the same.

I am inclined to believe that the HCO_3' ion is important in the regulation of respiration, and at this laboratory various methods have been employed during the past two years to arrive at the proof. The aug-

menting effects of intravenous injection of sodium chloride and sodium bicarbonate on pulmonary ventilation have already been mentioned. The effects of sodium bicarbonate on the rate of perfusion in the pithed frog (45) are somewhat irregular, but marked constriction which might be expected from increased alkalinity was not obtained. Lashmet (44) finds some indication of sodium bicarbonate increasing the acidity of the interior of the cells. The manganese electrode (46) placed in the tissues instead of in the blood indicates increased acidity on the injection of sodium bicarbonate. Rous and Drury (57) obtained the same results using indicator methods. Whether this increased acidity is an ionic effect or due to slowed circulation leading to asphyxial acidosis must be determined. The depression of hyperpnea of lack of oxygen by the administration of oxygen is perhaps a most important example of ionic exchange in the excretion of acid. At any rate it seems probable that the depressed respiration may be due to the great reduction of HCO_3' ions in the arterial blood resulting from the expulsion of carbon dioxide by oxidation of the hemoglobin in the lungs. This reduction should increase the diffusing gradient of the HCO_3' ions and lead to increased excretion of acid from the center.

THE OXYGEN TENSION OF THE ARTERIAL BLOOD AS A REGULATOR OF RESPIRATION. Following the work of Kussmaul and Tenner (1857) (58) indicating a chemical explanation of muscular cramps during cerebral anemia the mechanism of the stimulating effect of lack of oxygen became the subject of innumerable investigations. We can only hope to refer in part to this work simply using individual investigations as indications of the trend of progress or change of view.

Rosenthal in 1862 (59) proposed that lack of oxygen stimulated the center directly; Pflüger in 1868 (60) suggested indirect stimulation by circulating metabolites accumulating as a result of oxygen-lack, and Herman (1870) (61) proposed that oxygen regulated the excitability of the center to its normal respiratory stimulus, carbon dioxide. The solution of the problem of respiratory control seems to hinge on the peculiar effects of oxygen. For various reasons this phase of the subject has proved to be exceedingly elusive. A review of the literature indicates uncertainty and vacillation, and to-day we find these theories in modified forms all employed to explain the regulation of respiration.

The basis of Rosenthal's theory was the increase in oxygen tension of the arterial blood associated with apnea produced by overventilation of the lungs with room air. He believed that it was the overoxygenation of the blood which produced the apnea. Later work, however, showed

that similar oxygenation produced by the administration of pure oxygen during normal respiration exerted no noticeable effect upon pulmonary ventilation. And, likewise, it was demonstrated that overventilation of the lungs with hydrogen or nitrogen elicited apnea (62). There was no question then, that the proposed basis of the theory of direct action of lack of oxygen was unsound.

Pflüger in the meantime had proposed a theory of the indirect stimulation of lack of oxygen by the accumulation in the circulating blood of incompletely oxidized metabolites. In accordance with this theory the apnea following excessive ventilation was due to the removal of these products by oxidation in the lungs. But since he found that excess of carbon dioxide along with excess of oxygen also stimulated respiration, he then suggested that the normal carbon dioxide of the blood normally stimulates the medulla.

These products of incomplete oxidation which excite respiration were not clearly defined by Pflüger; so the accumulation of data on the formation of lactic acid under anaerobic conditions and its accumulation in the blood and urine led to a modified view of Pflüger's theory of indirect action based on the total acidity of the blood. Boycott and Haldane (63) concluded from their experiments on exposure to low oxygen pressure that the hyperpnea was a result of the combined stimulation of the center by the carbon dioxide and the lactic acid of the arterial blood. Geppart and Zuntz (64) were inclined to regard stimulation by such acid substances as lactic acid as wholly replacing carbon dioxide at very high altitudes. But Beddard, Pembrey and Spriggs (65) believed that these acids disappear too slowly to efficiently replace carbon dioxide as a regulator of respiration. Linhard (1911) (66) expressed the same objection.

Immediately following the suggestion of Boycott and Haldane (1909–10) of the response of the respiratory center to the total acidity of the arterial blood came the explanation of periodic respiration by the alternate formation and disappearance of lactic acid with fluctuations in the oxygen tension of arterial blood within the respiratory center itself (67), (68). These views were supported by Mathison (1910–11) (69), (70) by his experiments on the effects of asphyxia on blood pressure. This explanation served to meet the objections of Pembrey and Spriggs, and of Linhard, and seemed to offer a logical interpretation of the regulative action of oxygen; but with developments which were soon to follow the theory was dropped. For in 1912 the Pike's Peak Expedition (71) failed to reveal the production of lactic acid as indicated by

the blood at an elevation of 15,000 feet, and there was a return to the original view of Pflüger. In the report of this expedition it is stated that

> Henderson suffered from marked hyperpnea on rapidly squeezing a small india-rubber bag, though the exertion was very slight; at sea level this marked effect could no longer be obtained, though it was partly present at Colorado Springs. The most probable explanation is that products of imperfect oxidation in the fatigued flexor muscles of the fingers escape oxidation in the lungs in consequence of the lowered alveolar oxygen pressure at Pike's Peak, and were thus enabled to act on the respiratory center. This explanation is in harmony with our general conclusion as to the physiological action of low atmospheric pressures. The matter is at present being investigated by one of us as the phenomena are evidently of great interest.

About the same time the theory of direct stimulation of the respiratory center by lack of oxygen was vigorously defended by Loevenhart (72). The theory was supported on an entirely new basis. Loevenhart was impressed with the shortness of the latent period of the respiratory center to the injection of sodium cyanide. "This extremely rapid action precludes all possibility of explaining the stimulation as due to excess of CO_2 or accumulation of acid products. It proves beyond peradventure that the cells respond with stimulation to a decrease in their own oxidation." This view is accepted and supported by a number of workers (73), (74), (75), (76), (77).

In 1919 Haggard and Henderson, and Haldane, Kellas and Kennaway reported independently that the hyperpnea of high altitude is not due to increased hydrogen ion concentration of the blood because the concentration is actually diminished. Previous investigations having shown the diminution of the arterial carbon dioxide tension and the absence of increased lactic acid in the blood at high altitude left only one other stimulus to account for hyperpnea; namely, low arterial oxygen tension.

Following these observations Lutz and Schneider (76) found that the heart rate increased and the alveolar carbon dioxide decreased on reducing the barometric pressure to 656 mm., and that the oxygen consumption diminished at 530 mm. This extreme sensitivity of the medullary centers to a reduction of only 20 mm. of oxygen gave further support to the theory of the direct effect of lack of oxygen.

The occurrence of decreased hydrogen ion concentration of the arterial blood at high altitudes led Haggard and Henderson to discount the action of the hydrogen ions, and attribute hyperpnea to other factors.

In their paper on the Fallacy of Asphyxial Acidosis they call attention to the "paradox of anoxemic hyperpnea." They state,

> Several writers, Christiansen, Douglas, Haldane, Parson, L. J. Henderson, and others hold that oxygenation of the blood increases the acid property of hemoglobin and assists in driving out CO_2, and that de-oxygenation of the blood should lower C_H and lower the CO_2 tension at a uniform CO_2 content. As the respiratory center is acutely sensitive to alterations of C_H, the C_H of the blood should therefore depress respiration. In fact, however, acute oxygen deficiency for even a few seconds induces hyperpnea in most persons (cf. Lutz and Schneider).
>
> This paradox appears at present insoluble. To say merely that oxygen deficiency is itself a stimulus or that it governs the excitability of the respiratory center for CO_2 leaves the question as to how it does it quite unanswered. But evidently the stimulation of breathing in this case is not due to increase of C_H, for C_H is altered in one sense while activity of the respiratory center is altered in the physiologically opposite sense.

I offered an explanation of the "paradox of anoxemic hyperpnea" (78), showing that the reduction of hemoglobin produced by the administration of nitrogen must of necessity impair the transport of acid from the tissues, and thereby increase the acidity of the respiratory center itself despite the increased alkalinity of the blood. (For details see section on the dual function of hemoglobin.)

At the same time I presented evidence that lack of oxygen leads to increased tissue acidity from increased formation of acid as well as impaired transport of acid. And since the amount of acid formed in the tissues is a resultant of the anaerobic and aerobic production of fixed and volatile acids respectively, no latent period can intervene between the change in oxygen supply and the change in amount of acid formed. The direct experiments of Koehler, Brundquist and Loevenhart (79), reported at the same time, support the indirect or acid effect of lack of oxygen; for they find that lack of oxygen leads to the production of fixed acids which, they believe, begins at once.

When Haggard and Henderson concluded that hyperpnea of anoxemia was a result of something other than acid they proposed their theory of "respiratory X," expressing the view that the respiratory center is stimulated during lack of oxygen by incompletely oxidized metabolites which enter the blood stream. The validity of this view can be tested. I had shown (80) that hemorrhage and reinjection produce changes in the volume-flow of blood out of proportion to the changes in blood volume, from which it follows that the injection of inert solution after hemorrhage must increase the nutrient flow. On this basis the harmful and beneficial effects of hemorrhage and injection on salivary secretion,

contraction of muscle, and oxygen consumption of the body as a whole, were explained, reasoning that if Haggard and Henderson are correct the hyperpnea of hemorrhage should be augmented by the injection of gum-saline solution by virtue of the increased supply of "respiratory X" to the respiratory center. Depressed respiration occurred immediately. As a matter of fact, the acceleration of the undiluted blood containing the full amount of "respiratory X" is the most effective in reducing respiration. Obviously if "respiratory X" is a factor it is completely masked by more important factors.

Even though proof is wanting for the existence of "respiratory X," and though the evidence on the whole supports the indirect acid effects of lack of oxygen, it may be wise to look for other effects of lack of oxygen. The British Hemoglobin Committee of the Medical Research Council (6) expresses itself as follows: "Want of oxygen must then be regarded as having a direct effect on the respiratory center, and it does not matter whether we regard that effect as due to an actual stimulating property of oxygen deficiency or to an increase in the sensitiveness of the respiratory center to the hydrogen ion."

Means expresses much the same view. Agreeing that pulmonary ventilation is regulated by the hydrogen ion concentration of the arterial blood, he believes that the ventilation for purposes of oxygen absorption is ordinarily in excess; but under abnormal circumstances the organism responds to a threatened or impending oxygen shortage by virtue of a direct or indirect effect of oxygen.

Pflüger, Douglas, Geppart, and Zuntz, Haldane and others have also suggested that lack of oxygen becomes a regulative factor only under conditions of stress. The precise point at which lack of oxygen comes to the assistance of acidity, however, has never been indicated. Yet the more carefully the experiments are controlled the greater becomes the demonstrable sensitivity of the center (76) to reduction in the oxygen supply. Assuming the continuous occurrence of lactic acid in the respiratory center I (36) proposed the theory of the continuous regulation of respiration by lactic acid. Since the amount of lactic acid present in the respiratory center is a function of the oxygen supply, oxygen must accordingly act continually as a controlling factor regardless of its tension in the alveolar air. In accordance with this theory in the regulation by oxygen there is no sudden step from "normal" to "abnormal" conditions. Oxygen is always functioning but in varying degrees.

THE REGULATION OF RESPIRATION BY THE ACID METABOLISM AND ACIDITY OF THE RESPIRATORY CENTER ITSELF. I have considered the

theories of respiratory control relating to oxygen, the hydrogen ion and the specificity of carbon dioxide. The point in common to all of these theories is the view that the arterial blood acts as the vehicle for the *stimulating agent*, be it excess of hydrogen ions or carbonic acid, or lack of oxygen—and by virtue of one or more of these agents stimulates the respiratory center. Says Luciani (127):

> Most of the interpretations of respiratory rhythm suggested from Rosenthal onwards (Pflüger, Hering, Rosenbach, Burkart, Marckwald, Gad, etc.) start with the fundamental concept that not only nutrition, but also functional activity, is maintained in the respiratory centres by the blood circulating in them, which, when normally constituted, acts as an external stimulus, i.e., it contains stimulating factors such as carbonic acid or the other products of tissue consumption. This general theory is always based on the apnoea which inevitably sets in when these stimulating matters have to any considerable extent been removed from the blood, so that it no longer acts as an effective stimulus.

The inclination to accept the principle that the tension of the respiratory stimulants in the blood (by this I mean the oxygen, carbon dioxide tension, and the hydrogen ion concentration) is the prime factor controlling pulmonary ventilation leads me to ask the pertinent question, what is the source of stimulation of the respiratory center during hemorrhage? It is not increased tension of carbon dioxide of the arterial blood, because that is markedly diminished. There is a similar reduction in HCO_3' ions. It is not increased lactic acid in the blood, because the effect is immediate. It is not arterial hydrogen ion concentration ion, because the hydrogen ion concentration is markedly reduced. It is not the arterial oxygen tension, because hemorrhage increases the oxygenation of the blood. It is not "respiratory X," as has been shown. This leaves hyperpnea without a single recognized respiratory stimulant to account for it. These are after-thoughts and not the considerations which led me to advance the present theory. It was not the association of hyperpnea with the diminution of every known form of stimulation which forced me to question the prevailing theories of respiratory control, but rather the production of apnea in the absence of any chemical change within the arterial blood. I refer to the experiments done in conjunction with Mr. Capp and Mr. Foote (35), in which hyperpnea of hemorrhage was transformed to apnea by the acceleration of unchanged blood through the respiratory center.

We accepted the most prevalent view that respiration was controlled by the arterial carbon dioxide or hydrogen ion concentration, and used this as a basis for studying the effect of hemorrhage and subsequent

injection of gum-saline solution on the volume-flow of blood. We reasoned that if hemorrhage decreases the volume-flow of blood, and if the formation of carbon dioxide in the body continues at its normal rate, the blood should return surcharged with carbon dioxide and free hydrogen ions which on reaching the respiratory center stimulate respiration. Ignorant at that time of the increased alkalinity of arterial blood in hemorrhage the hyperpnea seemed to satisfy our postulate. We reasoned further that if the injection of gum-saline solution accelerated the volume-flow of blood out of proportion to the dilution of the blood the nutrient flow would be increased (80). The carbon dioxide carriers would circulate at a more rapid rate and consequently would be more lightly laden with carbon dioxide; the hydrogen ions of the blood reaching the respiratory center would be diminished and respiration would be correspondingly depressed. But the immediate cessation of respiration on the acceleration of unchanged blood, that is, blood which had already left the tissues, forced the conclusion that the metabolism of the respiratory center and transport of acid from the center are primary factors in the control of respiration.

The theory which developed from these observations has been presented in detail before and is here repeated in brief.

The respiratory center possesses an acid metabolism of its own.

The rate of formation of acid in the center and the rate of transport of acid from the center determine the acidity of the center.

Changes in the hydrogen ion concentration of the respiratory center rather than of the blood constitute the prime factor in respiratory control.

Since the supply of oxygen determines the absolute and relative amounts of lactic acid and carbon dioxide formed in living tissues, and since it controls the efficiency of transport and elimination of acid, it constitutes the normal and indirect regulator of pulmonary ventilation.

The effects of lactic and carbonic acid are additive. They are exerted indirectly by way of the blood from the tissues, and more directly through their formation in the respiratory center itself.

Diminished oxidation in the respiratory center leads to an accumulation there of the relatively poorly diffusible lactic acid in a relatively poorly buffered cytoplasm and lymph, no longer fully protected by the potential alkalinity of the blood—the dual function being disturbed by the diminution in the reduction of hemoglobin and the liberation of alkali as it passes through the center.

By virtue of its own metabolism and its extreme sensitivity to minute

changes in its own hydrogen ion concentration the respiratory center is sensitive to minute changes in its own oxidations, and, therefore, to changes in the tension of oxygen in the arterial blood.

The capacity of the center to respond to changes in the arterial carbon dioxide tension consequent on fluctuations in the general metabolism, however, must also be a factor.

THE METABOLIC RATE OF THE RESPIRATORY CENTER AND ITS RELATION TO RESPIRATORY CONTROL. If we are willing to accept that the respiratory center possesses a metabolism similar to that of other tissues it must follow that on the whole carbonic acid diffuses steadily from the center into the blood. Only under special conditions, where the carbonic acid of the arterial blood increases above that in the respiratory center, could the flow be in the opposite direction. Nevertheless, the general level of carbonic acid of the blood at all times determines the general level of carbonic acid in the center by virtue of the resistance which it offers to outward diffusion.

There are, then, four important factors determining the acidity of the respiratory center: the rate of acid formation, the rate of acid removal, the general level of acid in the cells, and the buffer value of the cellular cytoplasm.

It is, therefore, essential to have some idea of the rate of metabolism not only during conditions of rest—when the pulmonary ventilation is normal—but during conditions of augmented ventilation, when the respiratory center presumably is working harder. It is at present difficult to predict the exact significance of the rate of metabolism of the respiratory center. With an extremely low rate the acid metabolism would be correspondingly unimportant, that is, the fluctuations in the acidity of the center would be primarily a function of the composition of the blood. The center would then behave more like a physical system than a physiological system, having a definite energy exchange of its own. The higher the acid metabolism of the center the more important this factor becomes, and the less important becomes the acid back pressure of the blood.

On the whole there seems to be an inclination to ignore the importance of the metabolism of the center due perhaps to the opinion that the metabolism of nervous tissue is low. This is the veiw of Hill and Nabaro (81). Barcroft (82) also states that "such evidence as is at our disposal goes to show that the brain wants but little oxygen; that little, however, that it wants it wants very badly indeed. Complete deprivation of oxygen would abolish consciousness in a matter of a few seconds."

Roberts (83) believes that "the group of cells comprising the respiratory center has a comparatively low respiration."

It would be desirable to isolate the respiratory center or better still the various centers described by Lumsden (84) but that is impossible; and data must, therefore, be obtained from various sources—these data permitting us to reason by analogy.

Aby (85) and Craigie (86) give us anatomical evidence in their studies of the capillary circulation of the brain. Aby states that "At a given age in a given organ the relative number of capillaries in two regions is a certain index to the relative intensity of metabolic changes in those regions." Craigie found big differences in the capillary supply throughout the brain. The gray matter was more richly supplied than the white; the poorest gray was nearly one half as rich again in capillaries as the richest white. He made no comments on the vital centers, but neighboring nerve tissue showed very high capillary counts. In the light of Krogh's work (87) these findings must receive careful consideration.

Jensen (88) states that the volume-flow of blood through the brain is much greater than through muscle or any other part of the body. I have shown in various studies (89), (90), (91), (92), (93), (94), (95), that there is a close correlation between tissue metabolism and volume-flow of blood; in fact, this relation is virtually the basis of my theory of respiratory control.

Nerve cell endurance of anoxemia seems to offer further contributory evidence. Roberts' (83) experiments show almost immediate paralysis of the respiratory center on clamping the vessels to the brain. The endurance of prolonged anemia is discussed by Craigie: "If metabolic processes are not more active in the richly vascular regions than in the poor ones, we might expect that the cells in the latter would probably be the first to succumb to anemia. This, however, is by no means the case. The small pyramidal cells in the cortex survive only eight minutes, while the cells of the cord survive forty-five minutes or more." The observation of Gomez and Pike (96) that twenty to thirty minutes of anemia of the medulla is incompatible with recovery would rate the metabolism of the medulla fairly high.

Going to the more direct experiments—Baglioni (97) finds muscle and nervous tissue the seat of heat production, and that activity and oxygen have marked positive effects on heat production. MacArthur and Jones (98), in their determination of oxygen consumption of ground nervous tissue, found the oxygen consumption of gray matter

twice that of white. The metabolism of the medulla lay between that of the mid brain and cord. The tissue behaved like other tissue in showing a temperature coefficient of 1.8. Moore (99) found the rate of acid production in the intact living medulla of the frog to be about twice that of muscle. McGinty and Gesell (113) found a very rapid formation of lactic acid in the severed brain of the dog, indicating a normal rate of metabolism considerably higher than that of the body as a whole. Garrey (100) definitely showed that the cardiac ganglion cells of the Limulus liberated acid in proportion to their activity, as shown by the action of inhibitory nerve fibers and the effect of temperature. Both the rate of discharge and rate of formation of acid (101), (102) on increase of temperature corresponded with Van Toff's temperature coefficient for chemical reactions.

The accumulated evidence seems to point to a relatively high metabolism of resting nerve tissue, and that this metabolism is increased with increased activity of the cells. As we shall see the latter point may be of considerable significance in explaining various respiratory phenomena. Another point of equal significance is the nature of the acid metabolism with variation in oxygen supply.

LACTIC ACID AS A NORMAL CONTINUOUS REGULATOR OF RESPIRATION. From the observations of Fletcher (103), (104), (105), and of Fletcher and Hopkins (106) we know that the acid metabolism of the surviving muscle changes with lack of oxygen. Lactic acid appears in large amounts to disappear again with the removal of oxygen want. The quantitative experiments of Meyerhoff (107), (108) and Hill (109) indicated the nature of the disappearance of acid. According to them, lactic acid disappears in two ways, oxidation and return to the non-acidic precursor state. For every four, five, or six molecules removed only one is oxidized, giving rise to three molecules of carbon dioxide.

From these figures I arrived at conclusions (78), (36), which appear most important in explaining many respiratory phenomena and changes in the acid-base equilibrium of the body. It is obvious that the supply of oxygen diminishes the total acid present; that anoxemia is associated with a high rate of acid formation and oxygen plenty with the minimum rate. That is, the lactic acid which is normally oxidized to supply the energy for further removal of other lactic acid, which is continually forming, is not oxidized. Therefore, all of the lactic acid which is formed remains. The effects of variations in oxygen supply were schematically represented as shown here:

$$(1)\ \frac{3\ H_2CO_3}{60\ NaHCO_3} \quad (2)\ \frac{8\ H_2CO_3}{55\ NaHCO_3 + 5\ NaL} \quad (3)\ \frac{3\ H_2CO_3}{60\ NaHCO_3 + 5\ NaL}$$

$$(4)\ \frac{2.75\ H_2CO_3}{55\ NaHCO_3 + 5\ NaL} \quad (5)\ \frac{H_2CO_3}{65\ NaHCO_3}$$

If the first ratio represents the normal acidity of the tissue the effect of the accumulation of five additional molecules of lactic acid will be shown in the second ratio. Five molecules of base will be lost and five molecules of carbonic acid formed. Assuming that sodium lactate has no buffer properties whatever the acidity is represented by the ratio 8/55. Compare this with 3/60 and assume that this change has occurred within the respiratory center and the hyperpnea of anoxemia is largely explained. The purpose of the hyperpnea is to adjust the ratio to its proper value which is shown in ratio number three. A more correct representation of conditions is shown in ratio number four. That ratio can be maintained only by continued hyperpnea. Though the carbonic acid and the $NaHCO_3$ are reduced, the NaL remains; as a matter of fact it is the important element in the maintenance of the hyperpnea, for it results in a reduction of the denominator of the acid-base ratio which increases the effectiveness of the constantly forming carbon dioxide. On admitting oxygen at ratio three, ratio five results. Only one lactate ion is oxidized; this forms three molecules of carbonic acid, giving a total of six molecules. Five of these combine with the five sodium ions that have been liberated. Such a reaction should theoretically lead to increased alkalinity; apnea should occur, and, as is well known, this is the result of administering oxygen after oxygen want.

We have shown a similar apnea following anoxemia produced by hemorrhage on subsequent injection of gum-saline solution (35). When the anoxemia was severe enough, injection not only produced apnea but this was followed by a prolonged period of depressed respiration which gradually increased to normal. Curiously enough, this period of depressed respiration was accompanied by oxygen consumption considerably above the normal before hemorrhage. It is a fair assumption that the greater the consumption of oxygen the greater the formation of carbon dioxide. One of the first principles of respiration is that other things being equal pulmonary ventilation varies with the amount of carbon dioxide formed. If that is true, what variable has been introduced? During anoxemia with the accumulation of lactic acid the lactate ion replaces the HCO_3' and carbon dioxide is blown off. The

bicarbonate of the body is diminished and lactate is increased. What happens now when anoxemia is relieved? There is an abundance of lactate ions and of oxygen. These conditions are favorable to high oxygen consumption. The lactate ion is oxidized in greater amount and carbon dioxide formation is increased; but, as was pointed out before, there is a simultaneous liberation of alkali disproportionate to the carbon dioxide formed. This alkaline effect decreases respiration. The experiments of Barr and Himwitch (110) agree with this interpretation. Blood heavily laden with lactic acid from exercising muscles when circulated through the resting muscle loses lactic acid, and gains in free hydroxyl ions. Ronzoni finds the same.

I (36), therefore, felt justified in employing the same reasoning to explain the beneficial effects of the administration of carbon dioxide with oxygen in the resuscitation from carbon monoxide poisoning described by Henderson. In carbon monoxide poisoning the body becomes poor in the HCO_3' ion and CO_2 and rich in lactate. As in hemorrhage, when lack of oxygen is relieved, there is a relative retention of carbon dioxide. The administration of oxygen robs the body of free carbonic acid the moment it is formed by the synchronous liberation of alkali accompanying the removal of the lactate ions. Carbon dioxide, therefore, should be administered to provide the respiratory center with the stimulus of which it has been deprived.

This view has been confirmed by the experiments of Hill, Long, and Lupton in muscle lack of oxygen produced by violent exercise. They find a relative retention of carbon dioxide following exercise, and by quantitative determinations arrive at the relative amounts of lactic acid pushed back to the precursor state and the amount disappearing by oxidation. Their later ratios of lactic acid removed to lactic acid oxidized average 7.46 with variations from 4.96 to 9.70 (111), (112). These figures point still more to the importance of lactic acid in the control of respiration.

To be sure, we have reasoned only by analogy assuming that the lactic acid metabolism of nerve tissue is similar to that of muscle. Though reasons were given which lent security to the analogy, quantitative data are more satisfactory. Such have recently become available through the experiments of McGinty and Gesell (113). Normal unanesthetized dogs were decapitated and the head split with one stroke of a T-shaped guillotine. One half of the brain of each animal was promptly frozen in liquid air, the other halves were frozen from two seconds to thirty minutes later. Lactic acid determinations served to establish

the normal initial value, and the curve of rate of formation during lack of oxygen. The normal value was found to be 72 mgm. per 100 grams of tissue. During the first three minutes of oxygen lack the rate of formation is virtually a linear function of time, 20 mgm. per 100 grams of tissue per minute; the curve then rounds off to the horizontal within a few minutes. In terms of carbon dioxide this is equivalent to a metabolic rate about sixteen times the basal rate of the body as a whole. Assuming that the accumulated argument which I presented above for the high rate of metabolism of nerve tissue is correct, and arbitrarily rating the metabolism of the brain at three times the basal rate of the body, there is still an ample margin for markedly accelerated acid production during anoxemia. As a matter of fact, the figures of lactic acid production (McGinty and Gesell) and the ratio of $\frac{\text{L. A. removed}}{\text{L. A. oxidized}}$ in muscle (Meyerhoff and Hill) roughly indicate the rating of normal metabolism given above. These experiments of lactic acid accumulation in the brain give strong support to the theory of acid stimulation during anoxemia. They are the first experiments which warrant definite statements concerning the rate of acid accumulation in nerve tissue, and establish valuable facts for the interpretation of respiratory phenomena.

In that connection what can be said as to the rôle of lactic acid as a regulator of respiration under natural conditions? The possibility of lactic acid becoming a factor under conditions of stress has been alluded to, but on the whole the opinion up to the present time is, as Means (10) expresses it, that "ordinarily carbon dioxide plays the major rôle in regulating respiration and the rate of ventilation is adjusted to carbon dioxide requirements. For purposes of oxygen consumption alone the ventilation is ordinarily in excess."

Now the amount of lactic acid in surviving tissue is a function of two processes: its rate of formation, which is an anaerobic process, and its rate of removal, which is an aerobic process. The supply of oxygen, therefore, determines the point of equilibrium, that is, the amount and kind of acid, and the amount of buffer present in tissue—all of which determine its hydrogen ion concentration. There appear to be no reasons for believing that conditions differ in the living tissues of the intact animal. With that in mind I reviewed the literature to learn the probability of a lack of oxygen in muscle during life (114–126), and found abundant evidence indicating low oxygen tension. With a reduced supply of oxygen in muscle Verzar found a reduced consumption

of oxygen. I found that small hemorrhages reduced the basal metabolism of the dog and the efficiency of contracting muscle. Krogh concluded from his observations on capillaries that lack of oxygen in muscle was a frequent and probably a normal occurrence. The occurrence of lactic acid in blood under healthy as well as other conditions is now well recognized. Using these data, I reasoned again by analogy and suggested the normal occurrence of lactic acid in the respiratory center and its consequent rôle as a continuous regulator of respiration in the healthy individual under natural conditions of life. The experiments of McGinty and Gesell confirm these views completely. First of all they demonstrate a high normal lactic acid content of the brain; second, that lack of oxygen in the intact animal as well as in the severed brain leads to increased cerebral lactic acid content, and third, the results gave no indication of an intervening latent period between lack of oxygen and the onset of acid accumulation. Lack of oxygen seems to meet the requirements for quick and slow regulation of respiration under any conditions compatible with life.

THE DUAL FUNCTION OF HEMOGLOBIN AS A FACTOR IN RESPIRATORY CONTROL. The action of oxygen in expelling carbon dioxide from the blood is an old observation; and just as oxygen expels carbon dioxide into the lungs, reduction accommodates the flow from the tissues into the blood. The importance of oxygenation of the hemoglobin in expelling carbon dioxide from the blood was strikingly demonstrated in the living animal by Werigo (128), who found that on equal ventilation of the lungs, one with oxygen and the other with hydrogen, the carbon dioxide in the lung ventilated with oxygen was invariably 2 to 3 per cent higher. Since then the important work of Christiansen, Douglas and Haldane, Van Slyke, Henderson, Warburg and others has rapidly advanced this subject which has proven to be of greatest moment in the transport of the respiratory gases.

Christiansen, Douglas and Haldane (129) showed that the absence of reduction of hemoglobin in the tissues would be accompanied by an increased carbon dioxide pressure in the capillary blood of 18 mm. L. J. Henderson (130) showed that the hydrogen ion concentration of the blood would be three hundred fifty per cent greater in the absence of reduction. This is strong evidence indicating that the liberation of alkali by the circulating blood must be one of the most important factors in the control of respiration, yet a thorough search of the literature indicates that this phase of the subject has been entirely neglected. I presume this oversight is due to the fact that disturbances in the co-

ordination of the dual function failed to attract attention. If all goes well with circulation and ventilation, the higher the oxygen consumption by the tissue, the greater the flow of blood, and the greater the total reduction of hemoglobin, and thereby adequate acid transport is provided; but if for any reason oxygen consumption is reduced, and acid formation is increased, a broken coördination of the dual function of hemoglobin occurs.

Let us imagine conditions prevailing within the respiratory center during lack of alveolar oxygen. The center suffers from want of oxygen. The acid metabolism changes in favor of increased formation of acid. Reduced oxidations in the center mean diminished reduction of hemoglobin in the blood. The total result is increased acid and decreased acid transport—obviously a potent mechanism for increased acidity of the center.

This represents half of the disturbance in the elimination of acid from the body. Let us now follow the venous blood to the lungs. The venous blood arrives partially reduced and laden with acid. The failure of the blood to become fully oxidized in the lungs represents the other half of the disturbance of the dual function of hemoglobin. The carbon dioxide is not expelled as efficiently as before, more returns to the tissues; this impedes the diffusion of carbonic acid from the tissues to the blood, and the tissues turn more acid.

Henderson (12) states that the hyperpnea on the administration of nitrogen represents a paradoxical phenomenon.

> It is a well-established fact that there is an interaction of oxygen and carbon dioxide upon hemoglobin, by which the removal of oxygen enables the blood to carry carbon dioxide more easily; that is, at a higher pH. The blood is therefore presumably suddenly rendered much more alkaline than normal by the nearly complete removal of oxygen. Nevertheless hyperpnea is induced instead of apnea, as might be expected on the analogy of the effects of voluntary breathing. Evidently low oxygen does not act on respiration directly through pH.

From what is known of the acid-base equilibrium of the blood these changes in reaction of the arterial blood must occur, and the direct experiment with the manganese dioxide electrode (Gesell and Hertzman) show a marked increase in the alkalinity of the circulating blood on the administration of nitrogen. Synchronously with the arrival of the deoxygenated blood at the respiratory center hyperpnea invariably began. But far from offering contradictory evidence to the acid regulation of respiration, these observations may simply offer an example of the lack of correspondence between the acidity of the blood and

that of the tissues. It would seem to follow as a necessary consequence that if reduction of hemoglobin in the tissues facilitates the transport of acid from the tissues to the lungs by the liberation of alkali, a similar reduction in the lungs will bind the carbon dioxide more firmly and facilitate the transport of carbon dioxide from the lungs to the tissues. To be sure, the tension of carbon dioxide in the arterial blood is diminished by the administration of nitrogen, but the amount returning to the tissues is increased and carbonic acid is dammed back in the tissues. On the administration of oxygen all the conditions are reversed, the acidity and the carbon dioxide tension of the blood are markedly increased, but the moment the blood reaches the center apnea occurs. Other things being equal in the particular conditions under consideration, the greater the acidity and the greater the carbon dioxide tension of the blood the greater the resulting alkalinity of the respiratory center, and the greater the depression of respiration. To test the validity of this statement, it is only necessary to turn again to the direct experiments of Werigo (128), in which one lung was ventilated with oxygen and the other with hydrogen. The blood from the lung ventilated with oxygen, even though it returns blood to the tissues with the higher carbon dioxide tension and higher hydrogen ion concentration, serves the body the more efficiently in the transport and liberation of acid.

The depression of respiration with increased oxygenation of the blood may be due to a number of factors: in part to improved oxidations in the respiratory center, in part to the consequent increased liberation of base in the blood as it passes through the center, and in part to the decrease in the carbonic acid of the blood which occurs in the lungs. The increased tension of CO_2 and H_2CO_3 should, of course, hinder the diffusion of carbonic acid in these forms from the tissues to the blood. On the other hand, it should be remembered that virtually 95 per cent of the carbonic acid is carried to the blood in the form of HCO_3' ions. In the acute experiment the tension of this particular form of carbonic acid varies inversely as the oxygenation of the blood. Oxygenation of the blood should, therefore, lead to increased diffusion of the HCO_3' ion. Everything points to the importance of this factor, and supports the view expressed above (43) on the ionic excretion of carbonic acid from the tissues into the tissue fluids.

There are different varieties of broken coördination of the dual function of hemoglobin. The broken coördination may be confined to the tissue capillaries alone or to the pulmonary capillaries as well. In

hemorrhage the disturbance is confined to the tissues alone. Due to the lack of oxygen there is a disproportionate amount of acid formed as compared with the alkali liberated. The blood comes to the lungs heavily laden with acid but there it is ventilated more than normal. The same conditions prevail in many cardiac conditions and in anemia. With low alveolar oxygen tension or carbon monoxide poisoning, however, the disturbance occurs in both sets of capillaries. Both the loading and unloading of the corpuscles is hampered. In cyanide poisoning loading and unloading are also hindered due to the fact that the hemoglobin is more or less permanently oxidized. With lack of oxygen in the alveolar air the hemoglobin is more or less permanently reduced. Oxygen poisoning represents still different conditions. In this instance the tissues receive their full quota of oxygen from the plasma and the hemoglobin remains permanently oxidized. It is, of course, possible that the high oxygen pressure reduces oxidations in the tissues (142), (143), (144), (145), and that this may account for the hyperpnea; yet there can be no question about the impaired transport of carbon dioxide, as was pointed out before (36). Recent experiments with the administration of four atmospheres of pure oxygen have shown an accumulalation of carbon dioxide in the tissues.

Views on the relative importance of alveolar oxygen and carbon dioxide tension as regulators of respiration may be considered advantageously under the topic of the dual function of hemoglobin. We have already called attention to the regulation of the excitability of the center to its normal stimulus by oxygen. Here I wish to call attention to conclusions arrived at from determinations of alveolar gases at the breaking point under various conditions, and at the onset of respiration following apnea produced by forced ventilation. That apnea lasts longer the longer the period of forced ventilation indicated the importance of carbon dioxide; yet the prolongation of apnea by the filling of the lungs with oxygen at the end of forced ventilation indicated the significance of oxygen (17). Hill and Flack (146) found that the oxygen percentage decreased faster than the carbon dioxide increased during suspended breathing, which led them "to suspect that the breaking point is fixed by a fall in oxygen rather than by a rise in carbon dioxide," and they state that "Haldane and Priestley are correct in saying that normally respiration is regulated by the tension of the carbon dioxide of the alveolar air; but in cases of obstructed aeration of the blood diminution in oxygen tension is the more important agent." On the other hand,

Douglas and Haldane showed that periodic respiration could be elicited during very high alveolar oxygen pressure—120 to 130 mm. This is from 20 to 30 mm. higher than the normal pressure at sea level.

Recalling the views of Pflüger, Herman, Hasselbalch, Haldane, Douglas, Geppart and Zuntz, Means, Henderson and others, it is apparent that this brief survey represents the tendency to look upon the effects of carbon dioxide and lack of oxygen as two distinct identities. Pflüger states that normally the normal carbon dioxide tension of the blood serves as the regulator of respiration; Herman, Hasselbalch and Haldane state that oxygen regulates the excitability of the respiratory center to the normal stimulus carbon dioxide; Douglas states that at high altitudes "want of oxygen then becomes a permanent factor in the immediate regulation of the rhythm of respiration;" Means, after presenting the evidence that normally ventilation is in excess of the oxygen needs, states "We are brought, therefore, to the question, how under abnormal circumstances does the organism respond to a threatened or impending oxygen shortage?"

For the most illuminating experiments in this connection we must again turn to those of Werigo, who had many years before demonstrated in the living animal the expulsion of carbon dioxide from the lungs by oxygen. He concluded that more than one-half the carbon dioxide formed in the body is driven out by the action of oxygen in the lungs. How then is it possible to consider the two respiratory gases separately? They are indissolubly connected by virtue of their interaction in the dual function of hemoglobin and the effect of oxygen on the acid production in the tissues. In this connection I stated (36) "the distinct separation of the effects of oxygen and carbon dioxide seems to be a very involved problem, for oxygen tension and rate of oxidation determine the kind and sum total of acid formed, the transport of acid from and to the tissues and its elimination in the lungs. As a matter of fact, in the ultimate analysis it would appear that the rate of oxidation controls respiration by determining the efficiency of acid transport and the amount and kind of acid formed in the respiratory center and in the tissues as a whole."

The question of respiratory control always reverts to the same consideration. Is there one primary stimulus to respiration or do oxygen and carbon dioxide exert their own distinctive effects? Is carbon dioxide or hydrogen ion concentration to be considered normal, and lack of oxygen abnormal? If so, precisely at what point can we say that abnormal conditions have arrived and lack of oxygen comes to the sup-

port of carbon dioxide? For example, if normally the blood is overoxygenated, as suggested by Means, how can we explain the increased ventilation resulting from a very slight reduction in alveolar oxygen. I am thinking of Schneider's work on the effects of barometric pressure on pulmonary ventilation, and of Fitzgerald's work on the reduction of alveolar carbon dioxide with very small changes in altitude. To be sure, the effects of oxygen are small; nevertheless important, as they appear to be definitely established. But even more to the point, if the blood is overoxygenated under normal conditions of barometric pressure, how are we to explain the periodic respiration described by Haldane and Poulton when the alveolar oxygen pressure is 20 to 30 mm. higher than normal? Part of the answer is to be found in the dual function of hemoglobin. So long as hemoglobin is incompletely saturated, increased alveolar oxygen tension must expel more carbon dioxide from the blood, and, therefore, influence respiration. I should venture to say that if the blood is overoxygenated under normal conditions, then the body is also overventilated under those conditions so far as carbon dioxide is concerned. Until due recognition is taken of the potent effects of the dual function of hemoglobin and of oxygen on the total acid production, we shall look perhaps unnecessarily for accessory mechanisms of respiratory control.

THE ALKALI CONTENT OF THE BLOOD AND ITS RELATION TO RESPIRATION. The correspondence between blood alkali and pulmonary ventilation in experimental and clinical "acidosis," and in man at different altitudes are accepted observations. Commenting on this relationship in his recent review of the physiological regulation of the acid-base balance of the blood, Henderson (12) states

> that the relative volumes of air breathed for a given mass of CO_2 exhaled depend upon the blood alkali. If nearly the same pH is to exist in the blood, and if the alkali of the blood of Pike's Peak is only four-fifths of that of New York, then the breathing must be and is five-fourths or 1.25 as great at this altitude as at sea level. So it is the blood alkali which serves as the intermediate control of respiration. It sets the degree to which all the CO_2 produced in the body is diluted with air by respiration before it is exhaled from the lungs. Thus for any altitude all these functions and factors are determined by the amount of the blood alkali. It controls the volume of breathing per unit mass of CO_2 produced in the body, the alveolar CO_2 pressure, and the free carbonic acid of the blood.

This statement is not intended to explain the regulation of blood alkali but expresses in terms of alkali the accepted view that the hydro-

gen ion concentration of the blood controls respiration. This view we have seen is untenable.

Explaining the level of blood alkali, Henderson (12) states that "the blood alkali in turn is determined in normal persons by the barometer. Thus the oxygen pressure is the fundamental control. But how? What are the processes through which this factor slowly regulates all others? This problem will be dealt with from a strictly behavioristic standpoint." He states further, "I once applied this term 'respiratory X' to the unknown factor through which oxygen and alkali act. The value of this factor is the resultant of the antagonistic mass action of oxygen and alkali. But such a term as "respiratory X' is merely a confession of ignorance."

I am inclined to believe that the difficulty encountered in approaching this problem rests with the importance attributed to the acidity of the blood rather than to the acidity of the respiratory center itself. After all the blood is only the environment and as distances go in microscopic cells, semipermeable membranes and excursions of ions, a very distant environment at that. Not only may there be abrupt changes in acidity passing from the inside of the nerve cell to the immediate tissue fluid on the opposite side of the cell membrane, but another abrupt change from the tissue fluid on the outer side of the capillaries to the blood on the inner side. In addition there is probably a progressive decrease in acidity of the tissue fluid from the tissue cell to the capillaries.

Accepting the view that lack of oxygen increases the acidity of the respiratory center, which in turn increases pulmonary ventilation, we have a basis for explaining the alkali content of the body and body fluids.

The source of alkali ($NaHCO_3$) of the body is presumably the carbon dioxide formed in the process of oxidation. The amount and distribution of the bicarbonate in the blood and in the tissue must be a function of the chemical composition of the plasma, of the tissue fluids, and of the cellular cytoplasm and the properties of the membranes separating these fluids.

It is a fair assumption that the greater the amount of carbonic acid in the tissues the more bicarbonate will they contribute to the blood by ionic exchange, and the more CO_2 and H_2CO_3 by molecular diffusion. What is the effect of oxygen-lack on the carbon dioxide content of the tissues? First of all, it presumably increases the acidity of the respiratory center and all the tissues in the body. The denominator of the acid-base ratio in the tissues is decreased and the numerator is increased

by the formation of lactic acid. Let us say the function of the respiratory center is to care for its own hydrogen ion concentration. Pulmonary ventilation is increased until the 1/20 ratio is reëstablished by blowing off of carbon dioxide. This, of course, occurs throughout the body resulting in a general decrease of blood and tissue alkali. Reversing the argument, increased oxygen supply to the tissues reduces the ever present lactic acid and thereby increases the denominator of the acid-base ratio. Respiration subsides and the numerator next increases throughout the body as a whole. There is a general increase in the alkali.

Assuming that the respiratory center is functioning to provide itself with an optimum acid-base equilibrium (the oxygen supply being automatically controlled thereby), and assuming further that the needs of the center are in tune with the needs of the body as a whole, a relatively simple mechanism of acid-base control presents itself for further quantitative elaboration.

In this connection the experiments of McGinty and Gesell (113) on carbon monoxide anoxemia are of interest. The lactic acid content of the brain and blood was increased by the administration of carbon monoxide. Undoubtedly the lactic acid content of the entire body was increased. The increased lactic acid of the brain presumably supplies the stimulus for the blowing off of carbon dioxide, which thereby leads to a general reduction of sodium bicarbonate. Optimum oxidation in the tissues leads to a reverse condition, a maximum amount of carbonic acid occurring along with a hydrogen ion concentration compatible with normal function. Provided the excitability of the respiratory center is normal, high alveolar and arterial carbon dioxide, and high arterial alkali indicate optimum oxidation in the tissues.

According to these views it is not the alkali of the blood which is the dominant factor controlling respiration, as is further borne out by the effects of intravenous injection of sodium bicarbonate. It is virtually the alkali content of the respiratory center which determines the alkali content of the blood and tissues as a whole by its control of pulmonary ventilation.

How quickly equilibrium is established between the blood and tissues is still unknown. The failure to consider this point probably accounts for contradictory conclusions concerning the regulative action of the hydrogen ion. Take, as an example, the experiment of Hasselbalch (16), in which the acid-base equilibrium of the body was slowly changed by diet, and the acute experiments in which acid or alkali was rapidly

injected into the blood stream. Hasselbalch finding constant hydrogen ion concentration of the arterial blood concludes that respiration is controlled by the arterial hydrogen ion concentration. Mellanby (131), Scott (20) and others, on the other hand, produced large changes in the arterial hydrogen ion concentration with little or no effect on respiration. It is probable, at least, that in Hasselbalch's experiments on man the blood follows closely on the changes in the tissues, and, therefore, may represent the conditions obtaining in the tissues; whereas in the acute experiment the tissues do not follow the rapid changes in the blood.

THE QUESTION OF INDIRECT ACID EFFECT OR DIRECT EFFECT OF OXYGEN-LACK ON RESPIRATORY CONTROL. Rosenthal (59) believed that he showed the unimportance of carbon dioxide and the importance of oxygen tension in the regulation of respiration, and, therefore, proposed the theory of the direct stimulating effect of lack of oxygen. The basis of his theory was disproved and later evidence accumulated indicating that lack of oxygen led to the formation of fixed acids, accounting for the respiratory changes. But the experiments of Loevenhart (72), indicating a very short latent period intervening between the arrival of cyanide at the respiratory center and the increased ventilation, convinced him that the time was too short to allow for the accumulation of acid for stimulation. I have shown, however, that this view is untenable; that reduced oxidations and altered acid metabolism are simultaneous phenomena. The only basis of a theory along the lines suggested by Loevenhart is a greater sensitivity of the respiratory center to lack of oxygen than to excess of acid, but there is no evidence to support this view.

Nevertheless, the exceptions to the parallelism between pulmonary ventilation and acidity of the arterial blood seemed to require a theory of the direct action of lack of oxygen. Since hyperpnea of lack of oxygen could not be linked with any of the recognized mechanisms of respiratory control, it is frankly stated (British Haemoglobin Committee) (6) that "want of oxygen must then be regarded as having a direct effect on the respiratory center," leaving the mode of action an open question. Obviously this position must receive due consideration, for oxidations must carry with them a train of possibilities.

If the initiation of an impulse is the function of a difference in permeability in different parts of the nerve cell membrane (139), (140), any condition which affects the structure of that membrane should have an effect upon its function. Looking at the problem in this light the funda-

mental method of approach seems to be the logical method of obtaining a clear picture of the chemical control of respiration. Of course, this should be linked with some plausible theory of the factors involved. Such a combination should lead to rapid progress.

Awaiting the final solution of the problem we can only point out the possibilities. If lack of oxygen, other things remaining equal, produces increased acidity of the respiratory center and if a direct relation between respiration and the acidity of the center can be demonstrated, the evidence may be as strong for the direct as for the indirect action of lack of oxygen, but the probability would perhaps rest with the indirect effect. On the other hand, if it be proposed (72) that increased acidity decreases oxidations and thereby regulates respiration, the correctness of that postulate can be tested. If reduced oxidation is the primary factor then equal reduction, whether by lack of oxygen or by excess of carbon dioxide, should elicit equal stimulation. What is the evidence?

First of all attention might be called to the parallelism between respiration and rate of oxidation, supporting the direct effect of oxygen. Lack of oxygen leads to decreased oxidation and increased pulmonary ventilation. If this lack of oxygen has persisted long enough, as in the hemorrhage experiments described above, replenishment of oxygen by the intravenous injection of gum-saline solution increases the oxygen consumption above normal for a period of fifteen minutes or more. This increased metabolism is associated with a pulmonary ventilation lower than normal, which gradually increases as the metabolism decreases. It is recalled, however, that the respiratory changes are as explainable on the basis of hydrogen ion concentration as on the basis of direct effects. Further, it is virtually certain that increased oxidation in the respiratory center may be associated with increased respiration. The hyperpnea of fever and hot baths (29), (30), (31) in the presence of alkalemia may be in part explainable by the effects of temperature on the metabolism and activity of the respiratory center. Recalling the effects of temperature on the rate of discharge of the anterior horn cells (101), (102), and of the cardiac ganglion cells of the Limulus heart, as well as the increased acid production, suggests the mechanism. Even though there is sufficient evidence that oxidations are diminished by an increased hydrogen ion concentration, these findings do not preclude the possibility of increased oxidation in the presence of increased hydrogen ion concentration, for the concentration of the lactate ions is known to control the rate of oxidation as well. And so it seems probable that even though the recovery from prolonged anoxemia may be asso-

ciated with depressed ventilation and increased oxidation in the respiratory center, the hyperpnea of resuscitation with oxygen and carbon dioxide must likewise be associated with such increased oxidations.

A general comparison of the stimulating effects of the administration of air poor in nitrogen, and of the administration of carbon dioxide throws further light on the question. The most striking difference between stimulation by lack of oxygen and stimulation with carbon dioxide is the uncertainty of the effects of the first as compared with the certainty of the second. This has attracted the attention of many.

Loevenhart's (72) comments represent what has been generally observed.

> The effect of reducing oxidation in these centers depends on three factors: first, on the extent to which the oxidative processes are reduced; second, the suddenness with which they are reduced; third, the condition of the centers. If oxidation is reduced below a certain level, stimulation will not be observed. The more suddenly the rate of oxidation is reduced, the greater will be the stimulation. If the reduction of the rate of oxidation is very slow no stage of stimulation will be noted, as the irritability of the cells will then decrease faster than the stimulus increases. Finally, the better the condition of the center the more readily is the stage of stimulation demonstrable and conversely, the poorer the condition of the center the more difficult it is to elicit a stage of stimulation.

I have noted every grade of response from strong stimulation of respiration to strong depression with changes in oxygen supply, and have to a certain extent been able to supply the conditions for either response. The failure to fully appreciate the double effects of lack of oxygen may lead to opposite interpretation of results; take, for example, the difficulty of Roberts (83) in eliciting virtually nothing but depression of respiration in hemorrhage, and on ligating the cerebral arteries. Similar procedures in the hands of others lead to diametrically opposite results. Such are the effects of oxygen.

But what are the effects of carbon dioxide? An increase in alveolar carbon dioxide of 1.5 mm. is sufficient to double respiration (14). The effect is so constant that the method of carbon dioxide administration is employed for quantitatively testing the excitability of the center (147). Mathison states that when carbon dioxide fails to excite lack of oxygen also fails, but carbon dioxide may excite when lack of oxygen fails. Mellanby (131) found striking differences in response to lack of oxygen and carbon dioxide. "After the respiratory movements of an animal have been diminished or annulled by carbon monoxide a marked

stimulation of the respiratory center can be produced by carbon dioxide."

If lack of oxygen and excess of carbon dioxide act in the same way, how are the differences in effects explained? The necessary conclusion would seem to be that carbon dioxide produces stimulation by some effect other than reduction of oxidations. On the other hand, granting that reduced oxidations invariably lead to increased production of acids, why does lack of oxygen so frequently fail to stimulate? This might well be due to the general paralytic effect (132), (138) of oxygen lack on living tissue. We need only assume that lack of oxygen has two effects upon the respiratory center: a harmful effect upon the vegetative function, and an excitive effect upon the activity of the center. Since the activity of the center must undoubtedly be an energy consuming process, it would depend to a large degree on the disturbance of the vegetative function which provides the energy. This agrees very well with the general observations of Loevenhart that the better the condition of the center the more readily is the stage of stimulation demonstrated with lack of oxygen.

Though the evidence appears to indicate that a high rate of oxidation within the respiratory center may be associated with either depressed or augmented ventilation, and that a low rate of oxidation within the center may be associated with either augmented or depressed ventilation, and though the evidence seems to preclude the action of acidity by virtue of the accompanying changes in oxidation, it would be ridiculous in the present state of our knowledge to deny effects of oxygen other than the indirect acid effects. It would seem almost self-evident that the state of oxidation of the limiting and active membranes involved in the activity of the respiratory center would have some effect on the behavior of these membranes. One need only consider the effects of anesthetics (139), (140) and various salts on the respiratory center to realize that more than one factor plays a part in the control of respiration. We say that anesthetics depress the excitability of the respiratory center. During anesthesia there is lack of oxygen, excess of carbon dioxide and hydrogen ions; nevertheless, respiration is lower than normal. But, on the other hand, aside from the depression of activity, the center seems to follow the same general laws of response to respiratory stimuli.

The limited space prevents a discussion of the fundamental problems involved in the automatic and reflex activity of nerve tissue. It should be recalled that the regulation of respiration in its ultimate analysis may

be found to be an electrical phenomenon occurring with the aid of a surface membrane, and changes in composition of the fluids on both sides of that membrane. Whether acid is the only agent which can produce the necessary electrical disturbance involved in the nerve impulses is highly improbable; on the other hand, it may be the agent most employed in the body.

It is only fair in conclusion to call attention to comments on my theory which have come to my attention. Means (10), in supporting the theory of respiratory control by the hydrogen ion concentration of the arterial blood, states that a still more modern theory is that the reaction of the tissues of the center itself is the true stimulus. Roberts (83) states that "In view of the far-reaching conclusions which Gesell draws and the dubious nature of the experimental evidence upon which they are based I have thought fit to re-investigate the effects of haemorrhage on respiration." And further, "But the fact brought out that pulmonary ventilation does not vary inversely with the blood-pressure completely negatives Gesell's assertion and shows that his theory is without experimental foundation." Henderson states, "Such phenomena as these require to be considered, explained and harmonized before the behavior of the hemato-respiratory functions in health and disease can be regarded as solved. Such problems are not solved by merely referring them wholesale to disturbance of pH. Nor are they solved by transferring them to the respiratory center itself (Gesell)." Schneider, Truesdell and Clarke (148) believe that "This theory provides the best explanation for the respiratory variations of the type of anoxemia we have studied. According to it the early slow increase in minute volume of breathing is accounted for by a gradual accumulation of carbon dioxide and lactic acid in the center, because of a reduction in the rate of oxidation that occurs when the oxygen tension of the arterial blood is lowered, and by failure of the blood to supply the usual amount of alkali for the removal of acids."

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THE SIGNIFICANCE OF HEMOGLOBIN IN SUBMAMMALIAN FORMS OF LIFE

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Within the last twelve months the whole problem of "placing" hemoglobin in the chemistry of life has changed. A year ago one regarded hemoglobin as something which was well nigh universal among the vertebrates but which occurred only in sporadic ways and in unaccountable positions in the lower forms of life. All that is still true.

The change that has occurred is in the recognition of the fact that hemoglobin is itself only a special adaptation of something much more universal—very special but very important.

Let us commence then with the consideration of another substance, cytochrome, which appears to be of such wide occurrence throughout nature that in comparison hemoglobin only occupies a corner of the field which cytochrome covers. One might take up cytochrome almost anywhere. From the historical point of view, it was actually taken up by Keilin (16) in the study of the pigments of muscle. But then if you would woo cytochrome you must so far seek it where only it is to be found, namely, in the living tissue—or at all events in the tissue which still preserves its structural entity. Step across the line which separates biology from biochemistry and at once you find yourself—as MacMunn (21), (22) and a host of others have done—in the region of artifacts. Let us commence then with the spectroscopic study of the pigment in the muscle of the bee's wing, examined without any damage to the structure of the fibers. Here there is only one pigment which we will term cytochrome, it is that which MacMunn termed myohematin, and and probably his view of this pigment, if we confine ourselves to invertebrates, was not seriously in error. Let us define precisely what we are discussing. The pigment in the oxidised condition has so far as may be seen by an ordinary microspectroscope no spectrum but has in the reduced condition four bands, these may be termed a, b, c, and d and their positions of maximum density are as follows $a = 6046$; $b = 5665$; $c = 5502$; and $d = 5210$ Ångstrom units. They differ in density,

the most dense being band *c*. The following instructive demonstration shows the difference in density. Some fresh muscle from the bee's wing may be mounted for examination with the microspectroscope. If a cover slip be placed over, and in contact with the muscle, the pigment will be reduced and the four bands easily seen. The cover is now gradually pressed down, reducing the thickness of the layer of muscle: one band after another disappears, till at last *c* only remains. One may infer the ease with which confusion can arise. Were the pigment in the hands of one who had never seen it before he might describe it as having one, two, three or four bands according to the thickness of the layer through which he was observing it. On many occasions Keilin on first inspection saw only band *c*, but on none of these has an increase of the depth of the layer inspected failed to reveal all the four bands.

Unlike hemoglobin the bands are of constant position to within five Ångstrom units. More nearly than that they have not been measured.

One of the most remarkable points about this pigment is the range of life over which it occurs. Like hemoglobin it is to be found in most phyla of the animal kingdom in which there is any well-developed differentiation into organs, but whereas hemoglobin only turns up rather occasionally, cytochrome is of very frequent occurrence in almost every form of animal life that has been studied and in a great variety of organs. For instance, to take two snails, *Helix aspersa* and *Limnea stagnatis*, cytochrome is distributed through the bulk of the organs in both, but hemoglobin is confined to the muscles of the radula and stomach in *Helix aspersa* and is not found at all in *Limnea stagnatis*. For a complete list of the forms in which cytochrome is found, the reader must refer to Doctor Keilin's paper in the Proceedings of the Royal Society (16).

The most striking proof of the wide distribution of cytochrome is that it may be seen with ease in yeast, the onion, the bean and many other forms of vegetable life including certain bacteria. In yeast for instance if a layer of the moist substance about 2 mm. thick is illuminated by the transmitted light of a small arc and viewed with a Zeiss microspectroscope, the bands are readily visible. Attempts to isolate cytochrome would, I said, take us into the land of artifacts. Into that land we must now boldly go if we are to discover the relation between cytochrome and hemoglobin. Cytochrome appears to be an association of three hemochromogens or perhaps of two hemochromogens with something else, but we cannot let this statement pass without first informing the reader that the word hemochromogen has taken on a new meaning. It remains attached to the old spectrum—the thing which is extracted with soda or

potash from blood stained rags. The recent work of Anson and Mirsky (3) has however shown that the physiological world was under a complete misconception as to what that spectrum stood for. It stands in fact for a conjugated protein or a nitrogenous body of that general character, and only for one of many such. Let us therefore acquire a correct understanding of what we mean by a "hemochromogen."

Anson and Mirsky's work started from an attempt to find the greatest common denominator in the various forms of hemoglobin which are found in different animals. No two animals appear to possess hemoglobins which are spectrally quite the same, the bands being displaced a little in one or other direction. If however these various hemoglobins are treated with alkali and reduced, they yield spectra which are identical in so far as the positions of the bands go. How could hemochromogens which were the same yield hemoglobins which were different?

On the old theory of what hemochromogen was, the question was easy to answer; hemochromogen was held to be the non-protein nucleus of the molecule and the differences in the spectra were to be attributed to differences in the attached protein.

The above explanation probably is sufficient to account for the differences between different natural hemoglobins, but further investigation showed that many hemochromogens may be produced which are not obtained from vertebrate bloods, and which possesses spectra that are not identical.

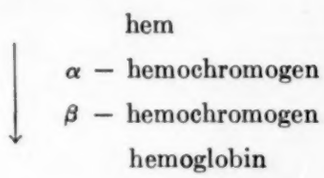
The following is a summary of the principal facts which form our present conception of hemochromogen.

Hemochromogen i.e., the substance which gives the "hemochromogen spectrum," according to the work of Anson and Mirsky (3) (and their work, is the basis of my information on this subject) is not a protein-free body, but a body of the same sort of structure as hemoglobin but of a simpler nature. The oxide of hemochromogen, hematin is also a conjugated protein.

2. When hemochromogen and hematin are stripped of their protein moieties, two bodies are obtained with diffuse spectra, these may be called reduced hem and hem respectively. They are the bodies obtained when hemin is treated with NaOH. Reduced hem is obtained if a reducing agent is present, but in the presence of oxygen hem is obtained. The substance to which Hoppe-Seyler gave the formula $C_{34}H_{35}N_4FeO_5$ is hem.

3. Hemoglobin may be synthesised in the following way. Starting from hemin, which is the chloride of a hypothetical base to be referred

to as hem, the base may be liberated by the addition of NaOH. This base is then reduced giving a body which has a spectrum quite different from that of hemochromogen but when globin is added a product is formed which though hemochromogen as we understand it, we may refer to as α-hemochromogen. α-hemochromogen is transformed in β-hemochromogen, (the familiar body) by the addition of further globin. By adjusting the hydrogen ion concentration β-hemochromogen passes into reduced hemoglobin. We are therefore confronted with a series of four different spectra:



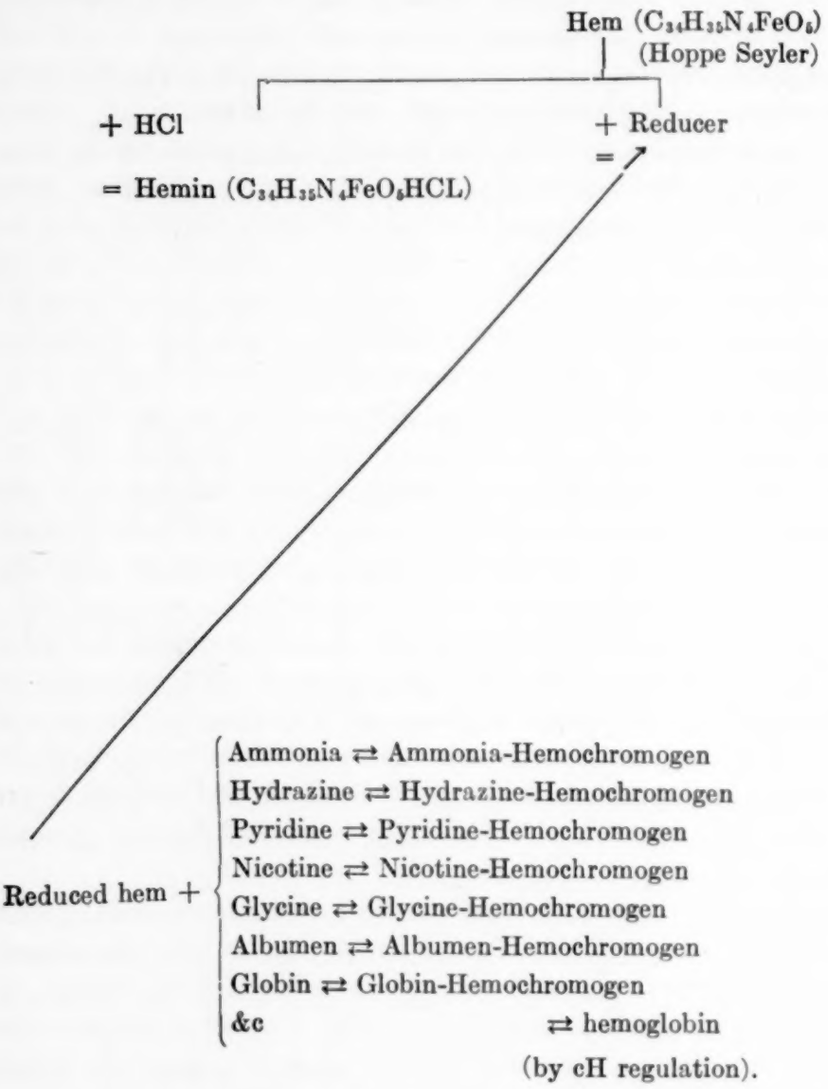
4. So long as the protein body is a globin the spectral bands of the hemochromogen formed are in a constant position and for this reason the hemochromogens of all mammalian bloods give bands of identical wave length.

5. But hemochromogen made from globin is only a special case of a wide series of compounds of the same general type: albumen may be added to the reduced hem instead of globin, or much simpler nitrogenous compounds, nicotine, pyridine or even ammonia. When one so extends the series using widely different nitrogenous moieties one finds that the position of the spectral bands alters with the nature of the nitrogenous body used.

6. Hemochromogens then are compounds of a substance hem which has some such formula as $C_{34}\ H_{35}\ O_3\ N_4Fe$ with one or other of various nitrogenous compounds. These last may embrace a great range of substances from ammonia to albumen. The hemochromogens are maintained as the result of balanced actions, there being always some hem and some of the free nitrogenous body present as well as the hemochromogen.

7. The failure to realise that there was an intermediate body between hemin and hemochromogen is partly due to the way in which hemochromogen is often made. If hemin in their presence of a reducing agent, is treated not with NaOH, but with ammonia or hydrazine, not only is the base (hem) liberated but it unites at once with this ammonia or hydrazine to form this corresponding hemochromogen and thus the hemochromogen spectrum at once appears.

The following scheme represents the relationships which I have indicated:



[The names acid and alkaline hematin, like hemochromogen are best kept to represent the bodies possessing the spectra to which those names have hitherto been attached. These bodies are conjugated proteins corresponding to the hemochromogens but more highly oxidised. The body to which Hoppe-Seyler gave the formula $C_{34}H_{35}N_4FeO_5$ to which the name hem is now given, has a much more diffuse spectrum.]

We have seen what the term hemochromogen signifies at the moment, and incidentally we have seen its relation to hemoglobin. Let us go back and take up cytochrome where we left it. We had emphasized the

fact that attempts to isolate it have so far only resulted in the production of hemochromogens of which apparently three (or possibly two in conjunction with a kindred body which is not a hemochromogen) contribute to the "make up" of cytochrome.

We have said that cytochrome ordinarily shows four bands *a*, *b*, *c* and *d* and that *d* may be resolved into three, we will now call these three a^1, b^1, and c^1. It seems probable that of the three substances which may be derived from cytochrome one is responsible for bands *a* and a^1, one for bands *b* and b^1, and the third about which little is known for *c* and c^1 (16). From among these may be obtained at least two substances very similar to hemochromogen, and which yield hem identical with that derived from other sources (4). The other substance also resembles hemochromogen.

Having traced the descent of cytochrome and hemoglobin, there remain some minor pigments to be fitted into the scheme.

Helicorubin. Helicorubin which was described by Krukenberg as occurring in Helix pomatia appears to be a concrete example in nature of a substance which is analogous to hemochromogen. The analogy traced by Anson and Mirsky (5) at least consists in it possessing the following properties:

1. The spectrum is of the same pattern but the bands are redwards of those of hemochromogen.

2. In many artificial hemochromogens the bands are shifted in precisely the same way.

3. The nitrogenous moiety may be detached from the hem in both helicorubin and hemochromogen and replaced by ammonia, in which case compounds identical spectroscopically are obtained.

Previous workers (MacMunn, Krukenberg, Dhéré) have pointed out the difference between helicorubin and hemochromogen prepared from mammalian blood and being unable to place it have called it a pseudo-hemochromogen. Here for the moment we must leave the matter until a more exhaustive enquiry has been made into the identities of the hems which form the basis of helicorubin and hemoglobin respectively.

An interesting property of helicorubin is furnished by the effect on it of alteration in the hydrogen ion concentration. In acid solutions it forms a reversible compound with oxygen, in alkaline solutions its affinity for oxygen is practically negligible (5). In this respect helicorubin has one analogous compound among the hem derivatives namely pyridine-hemochromogen (3). Redfield and Hurd (23) have shown that hemocy-

anin (which is of course a copper compound and therefore not a derivative of hem) can be of two kinds one of which has the affinity for oxygen increased and the other has the affinity for oxygen diminished by increased hydrogen ion concentration.

Chlorocruorin. While helicorubin appears to present an analogy to hemochromogen there is another pigment, chlorocruorin, which is analogous to hemoglobin. This is a green substance dissolved in the blood plasma of certain polychaete marine worms. It has recently been investigated by H. Munro Fox (11). The pigment exists in an oxidised and a reduced state with absorption spectra analogous to those of oxy- and reduced hemoglobin but pushed far to the red, to a distance much greater than the differences in wave-length between specific hemoglobins. While chlorocruorin forms a dissociable compound with oxygen, it has a lesser affinity for this gas than has any hemoglobin. This affinity is influenced by changes in hydrogen ion concentration in the same way as in the case of hemoglobin.

From chlorocruorin derivatives have been prepared corresponding to methemoglobin, hematin and hemochromogen. Since the hemochromogens prepared from various hemoglobins are spectroscopically identical it is interesting to find that this derivative of chlorocruorin is not identical with these: again its bands are shifted over to the red.

Now from such different substances as hemoglobin, helicorubin, actiniohematin, and cytochrome, hems have been prepared which when united with ammonia are spectroscopically identical. It is therefore remarkable to find that chlorocruorin gives a different hem, the ammonia-hemochromogen of which is not the same as those just mentioned. Hem is an iron-pyrrol compound. When the iron is removed a porphyrin remains. The fact therefore, that the hem of chlorocruorin is different from that of all the pigments mentioned above might be due to 1, the metal being other than iron; or 2, iron being united to a different porphyrin. The second alternative is the correct one. Chlorocruorin contains iron but has a porphyrin different from hematoporphyrin.

Actiniohematin. Actiniohematin, a pigment described by MacMunn, in certain actiniae appears to be in the *same category* as helicorubin. If the actiniae are ground up, dried and treated with ammonia for sufficient time their actinohematin yields typical ammonia—hemochromogen (4).

THE FUNCTIONS OF HEMOGLOBIN AND ITS RELATED BODIES. The differentiation of function amongst the compounds which hem makes

with nitrogenous bodies is very striking. In the cases both of cytochrome and hemoglobin the functions are bound up with oxidative processes.

Hemoglobin as a catalyst. Cytochrome appears to be preëminently a catalyst.

This claim which at present rests on purely circumstantial evidence, is as follows (16):

1. Judging qualitatively, cytochrome occurs in greatest concentrations in the muscles which are most active.

2. The more active the muscle has been immediately before death, the more quickly the reduced cytochrome band flashes out if under anaerobic conditions.

3. The quantity of cytochrome present corresponds to the intensity of the peroxidase reaction.

4. The presence of minute traces of potassium cyanide prevents the cytochrome taking up oxygen even when a sufficient supply of that gas is available.

Such then is the pigment in the wing muscle of the bee which MacMunn termed myohematin. It seems probable that when he extended the name to the pigments in vertebrate muscle he unconsciously used it to cover more than one substance, and confused it with another substance which has been known under many aliases, the myoglobin of Günther (12), myochrome of Mörner and muscle hemoglobin of Külma. From these it is quite distinct, though it may occur side by side with them in the same tissue. To them we must now turn.

The hemoglobin of muscle, say in the rat, is different from the hemoglobin of rats' blood not by more than the hemoglobin of one blood differs from that of another. Indeed the hemoglobin of rats' muscle very closely resembles that found in the larva of Gastrophilus (16). When in dilute solution it is much more easily seen in the oxidised than in the reduced condition, just as the myohematin is more easily seen in the reduced than in the oxidized condition. Thus an interesting illusion takes place; when a mixture of myohematin from yeast and hemoglobin from gastrophilus, is alternately oxidised and reduced the microspectroscopic appearance is that of an alternation between the bands of gastrophilus oxyhemoglobin and reduced myohematin. But the impression produced is that of a single substance which has bands of one character in the oxidised state and of another character in the reduced state. This in fact is what may be seen when muscle is oxidized and reduced under the microspectroscope and is the cause of much of the confusion

which has arisen about the supposed pigment which muscle contains. If, however, the mixture be reduced, a trace of KCN added, and the mixture then be exposed to oxygen, the two spectra will appear, the oxyhemoglobin which is not affected by potassium cyanide and the cytochrome which remains reduced even in the presence of oxygen because of the KCN (16).

What sort of system is presented by the combination of cytochrome, the catalyst in muscle, hemoglobin of unknown function in muscle and hemoglobin the carrier in blood, we do not know. It may be that the hemoglobin in muscle has a greater affinity than that in blood for oxygen and that it in its turn transfers the oxygen to the cytochrome, but that is pure speculation.

The position of cytochrome as a peroxidase of very wide distribution is supported by a considerable body of evidence and as far as is known it has no other function in relation to oxidation. The question naturally arises, can hemoglobin act as a catalyst and if so has it in fact any such action? That hemoglobin can act as a catalyst is shown by the recent work of Miss Robinson (25) in Professor Hopkins' laboratory. She has shown that in the presence of hemoglobin linseed oil shaken in a differential blood gas apparatus becomes oxidised much more rapidly than if the hemoglobin is not there and further, methemoglobin and hemin have the same catalytic property but hematoporphyrin has not, so that she attributes the catalytic action to the presence of the iron molecule. Iron in the inorganic condition is not without a similar effect but it takes place on a much smaller scale. The action unlike many is not inhibited by potassium cyanide.

Whether hemoglobin does act as a catalyst in the body is a question on which no final statement can be made at present, yet one may in this connection put forward certain general considerations. They divide themselves into 1, those which relate to hemoglobin in the blood; 2, those with regard to hemoglobin in the tissues.

1. It is the universal, and no doubt the correct belief that in the mammal the waste products of the tissues are oxidised where they are formed and not thrust into the blood for oxidation.

It must not be thought that I am throwing any doubt on this article of the physiological creed if I point out that the evidence for it is none too strong. It rests on the classical work of Pflüger showing a, that the venous blood gains as much, or nearly as much in CO_2 as the arterial blood loses in oxygen, and b, that little further change takes place if the blood be kept standing. The proof fails of being rigorous inasmuch as it

leaves out of account the possibility of oxidation taking place in, and being complete in the capillary. But if Pflüger's view is not quite proven it is at least quite reasonable, whilst the opposite view is quite unreasonable. If the question is whether Mahomet should go to the mountain or the mountain to Mahomet—well, of the two the prophet is the more mobile. It is not likely that a molecule to be oxidised, presumably large and sluggish, should diffuse in succession from the cell, through the lymph, the vessel-wall the plasma and finally into the capillary in less time than oxygen would take the same journey in the opposite direction. Therefore in such a system one could only conceive of hemoglobin acting as a catalyst, if molecules were being oxidised which resisted oxidation by another mechanism. Such a scheme is not impossible but it is very unlikely. It is most probable that the hemoglobin of the mammalian corpuscle never comes in contact with the material to be oxidised and therefore in the corpuscle at least the pigment in no wise functions as a catalyst.

The general tendency as one descends the animal kingdom is for the hemoglobin to get nearer to the cell. In many of the marine and terrestrial worms the hemoglobin is carried in as definite a circulatory system as in the mammal, dissolved in the plasma, and probably the oxygen pressure in the mixed blood which goes to the muscles is not more than a fraction of a millimeter. The possibility of oxidisable products getting into actual touch with the hemoglobin and reducing it by double decomposition is much greater in the worm than in the vertebrate. That is to say, if the oxidisable product is X, there might occur not

$$HbO_2 = Hb + O_2 \text{ in the blood corpuscle}$$
$$O_2 + X = XO_2 \text{ in the cell}$$

but

$$HbO_2 + X = Hb + XO_2 \text{ in the blood}$$

There seems to be at least the possibility of such a reaction.

2. Naturally the most likely site on which hemoglobin can function as a catalyst is that where it is in most intimate contact with the materials to be oxidised and one may probably say that if hemoglobin does not exert the catalytic properties in muscle it is not likely to do so at all. One therefore turns to muscle for some evidence that hemoglobin may act as a catalyst.

Hemoglobin as an oxygen carrier. The speculation as to what properties nature has gradually conferred upon hem in order to produce

the wonderful material hemoglobin is one than which few can be of greater interest, for, as we have shown in our previous article, on the properties of hemoglobin depends the existence of the higher creation.

First, hem must acquire some suitable nitrogenous substance. By "suitable" we mean a nitrogenous moiety for which

1. It must have a great affinity
2. With which it can form a very soluble hemoglobin[1] and
3. One which has the power of combining reversibly with oxygen.

These properties may be considered one by one (3).

1. The problem of affinity for the proteins. Regarding the union between hem and amino acids as a balanced action, it is found that the various nitrogenous bodies which have been tested have very different affinities for hem. On such a scale globin stands very high. The presence of hemochromogen therefore demands but very small quantities of free hem and free globin.

2. The problem of solubility. The utility of hemoglobin as an oxygen carrier depends upon its solubility. In this respect the protein, as Anson and Mirsky have pointed out, exerts a great influence. Hemin and its base hem, are both extremely insoluble. The general rule is that their compounds on the α-hemochromogen level are more soluble and on the β-hemochromogen level more soluble still. Yet as compared with hemoglobin most of these substances are very insoluble. Of the whole series, only a few of the compounds of hem which have been tested are soluble in a high degree; of these the globin compound is the most conspicuous. One may therefore recognise a double influence of globin in the evolution of a soluble oxygen carrier.

a. The general effect of all such nitrogenous bodies, and

b. The very remarkable specific effect which gives hemoglobin a solubility, and therefore, a virtue as an oxygen carrier, which the compounds of hem as a whole do not possess.

It is not by chance that hemoglobin carries the particular protein with which it is provided.

3. Nevertheless hemochromogen lacks one essential quality, namely, that of uniting reversibly with oxygen. How nearly it comes to possessing this quality is shown by the fact that it unites reversibly with carbon monoxide. Anson and Mirsky (3) have investigated this reaction and shown that the equilibrium curve is a rectangular hyperbola, of just the sort which oxygen would make with hemoglobin. Only within certain limits of hydrogen ion concentration does hemochromogen

[1] *d* can be resolved by more powerful instruments into three bands.

become hemoglobin and acquire the power of uniting reversibly with oxygen. What the essence of the alteration is we do not know. Hemoglobin may be a condensation product of hemochromogen, or again it may be a compound in which the hem is united to the globin in some different way from that of the hemochromogen linkage.

With regard to the possibility that hemoglobin is a condensation product of hemochromogen, the recent work of Adair (1) on the osmotic pressure of hemoglobin is of great interest. Adair has been dialysing samples of hemoglobin in dilute solutions for many months in the Low Temperature Research Station at Cambridge, at +0.6°C. and his results have convinced him that the molecular weight of hemoglobin is in the region of 68,000; that is to say, that it is four times the possible minimum—four times the amount which would contain one atom of iron. The possibility exists that the molecule of α-hemochromogen contains a single atom of iron and has an approximate molecular weight of 16,800, that of β-hemochromogen two or three such molecules and that of hemoglobin four such. Possibly by the time these lines appear the matter will be settled. The evidence so far as it goes at present suggests that the molecular weight of hemochromogen is less than that of hemoglobin.

When the actual difference between hemoglobin and hemochromogen is discovered, it will be most interesting to see how that difference renders the oxides so unstable as to break down in the presence of a reduced partial pressure of oxygen.

When one reflects upon the gas binding properties of hemoglobin in the light of the architecture of the hemoglobin molecule one sees clearly that the fact of hem forming a reversible compound with oxygen depends upon two things: 1, on its being united with a protein-globin, and 2, the union taking place at a certain hydrogen ion concentration. It is not surprising then to find that affinity for oxygen depends 1, upon the precise nature of the globin; and 2, upon the precise hydrogen ion concentration, associated perhaps with kindred environmental conditions such as the concentration of other ions, and of course the temperature. Thus we may distinguish two groups of conditions by which the equilibrium constant of the reaction between oxygen and hemoglobin may be influenced: 1, the chemical constitution of the molecule; and 2, the environment in which it is placed.

The study of those two sets of conditions must form the physicochemical basis of the evolution of the respiratory function of the blood —the means by which nature has adapted hemoglobin to the needs of

the various forms of life—or, to put the matter more correctly, has expanded the possibilities of life by the specialisation in various directions of the hemoglobin molecule.

The study of the chemical basis of evolution must then consist in

1. A study of the chemical nature of the hemoglobins in various forms of life, under identical and basal conditions.

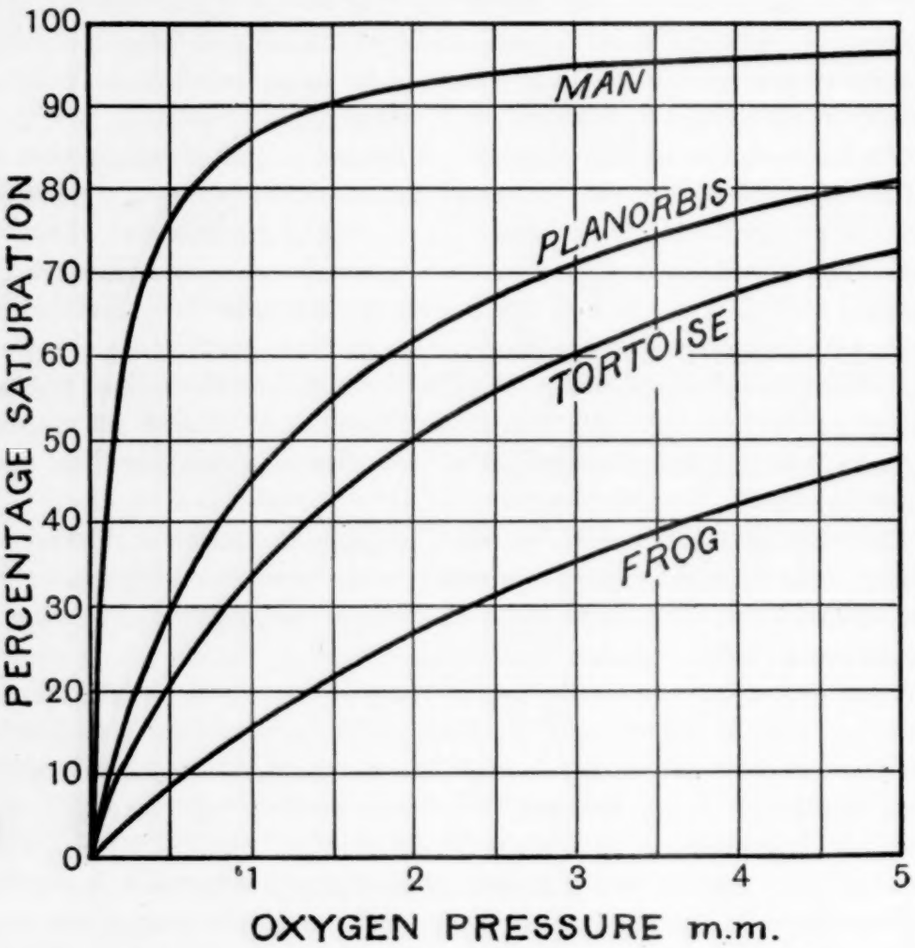


Fig. 1. Approximate dissociation curves of dilute hemoglobin from man, Planorbis, tortoise and frog at 12°C. and pH 7.4.

2. Having established the fundamental properties of hemoglobin as such, study must be made of the effect of environment upon each.

THE IDENTITY OR DISPARITY OF HEMOGLOBINS. Let us see what difference, if any, we may discover between the gas binding properties of the hemoglobins, as distinguished from the bloods, of various forms taken over a wide range of the animal kingdom. As an arbitrary means

of obtaining uniformity of conditions we shall take in each case solutions of hemoglobin obtained by laking the corpuscles (if the hemoglobin is in corpuscles) with saponin diluting to approximately one part of hemoglobin in 2000 of water, and buffering to 7.4 pH with phosphate mixture, and at 12°C.

With regard to the specific point: is the gas combining power of the hemoglobins of all forms of life the same? The answer is given by a

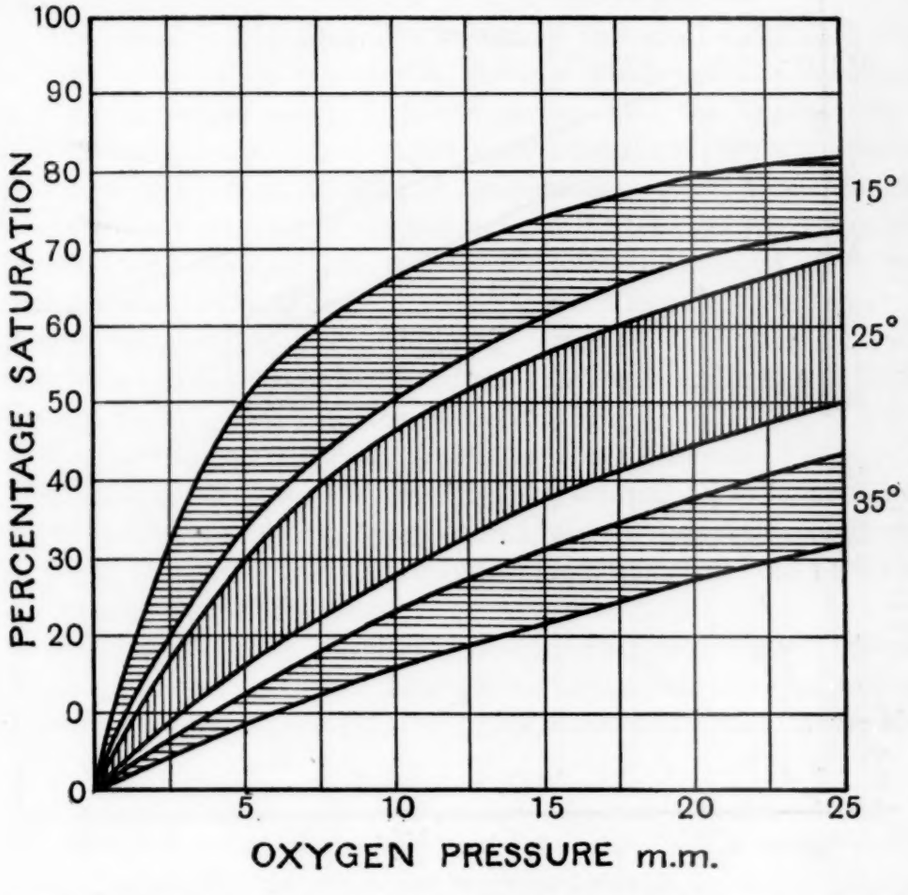


Fig. 2. Showing the variations within which the dissociation curve of frog's hemoglobin was found to lie at different temperatures.

recent research by Seliskar and Maçela (20) conducted under the above conditions.

The result of this research may be expressed by figure 1. The rectangular hyperbola which represents at least approximately, the equilibrium between oxygen and hemoglobin for dilute solution is given for the following forms: man, frog, tortoise and Planorbis. The curves are

only intended to give an indication of the sort of differences which exist, because only those on the frog and planorbis are the result of averaging a large number of individuals. How great the individual differences may be are shown by the extreme limits which so far have been obtained from frog's blood. These limits are shown in figure 2. At present it is not possible to say much about the philosophical ques-

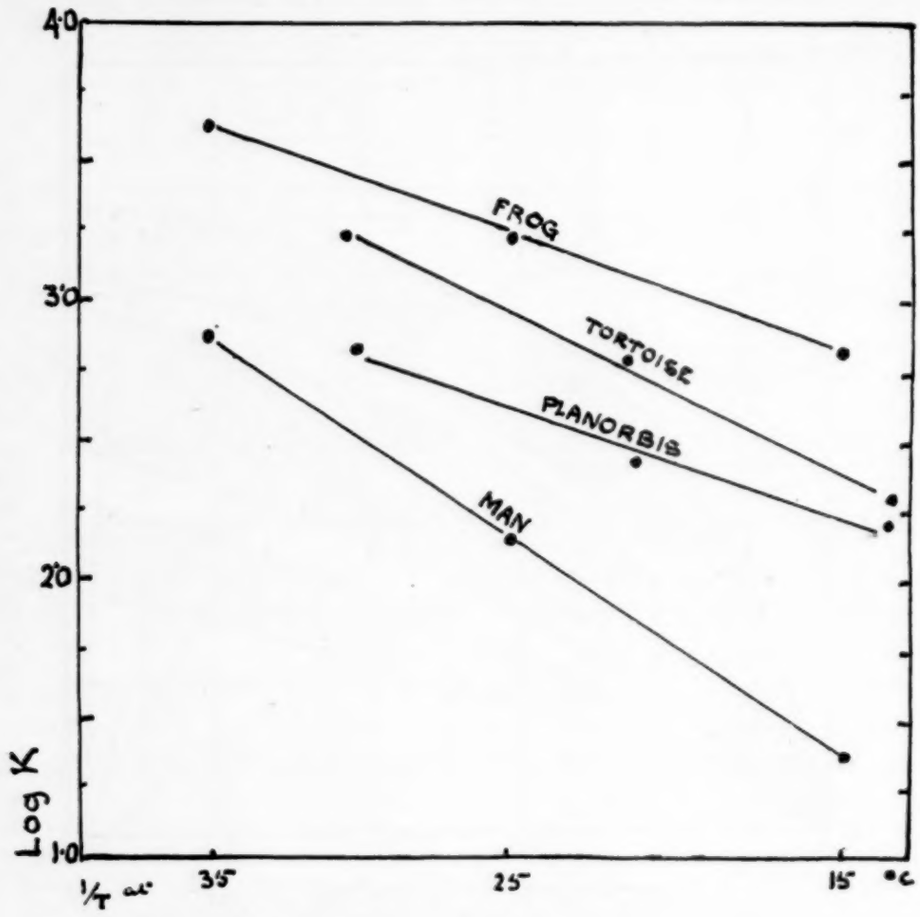


Fig. 3

tions which are bound up either with the great differences in species, or the perhaps more surprising dispersion within a species.

Thus the hen and the man, both warm-blooded, have hemoglobins of very different oxygen affinity at 12°C. The hen is well within the limits for the frog—both having nucleated corpuscles, but on the other hand proteus is more like man. With regard to a possible correlation between the size of the animal and the dissociation curve, the cat and the hen are

of the same order of size though their curves are far apart. With regard to activity, whilst it may be conceded that the snail and the tortoise which have almost identical curves are the very types of slow movement, the fowl and the cat whose curves approximate to those of man and the frog respectively are capable of great activity.

The dispersion within the species offers an interesting subject not only for speculation but for research, as there are few chemical variables which have been studied from the point of view of genetics.

In the endeavour to trace the process of biological adaptation from the basal nature of the hemoglobin, perhaps the first step is to consider the effect of temperature. It is not merely that the hemoglobins in different animals, and indeed in the same animal at different times are called upon to function at different temperatures, but it seems that not all hemoglobins respond in the same degree to temperature. Figure 3 shows the temperature coefficients for the average hemoglobins of frog, tortoise, Planorbis, man.

These temperature coefficients vary greatly. The largest is the human one and is over 5, whilst that of the frog is under 3, and that of Planorbis may be even less than that of the frog.

The former agrees fairly well with other computations.

One can say at present that when one compares the hemoglobins, reduced to their simplest terms (i.e., in dilute solution, buffered equally and at the same temperature) from various forms of life one finds that these hemoglobins differ greatly.

It was pointed out by Krogh (17) that the very sensitiveness makes it difficult to picture the same hemoglobin in the cold-blooded animals. According to Hill and me (8), the equilibrium constant of the reaction

$$Hb + O_2 \rightleftarrows HbO_2$$

had a temperature coefficient of about 3.5 to 4.5, that is to say, at 7°C. the oxygen would stick sixty-four times as tenaciously to the hemoglobin as at 37°C. Krogh calculated the coefficient to be about 5 from some other data of mine. Such tenacity seemed to him inconsistent with the efficient yielding of oxygen to the tissues. Two possibilities then presented themselves. Either Hill and I had over estimated the temperature coefficient, or the hemoglobin of warm-blooded animals was different from that of cold-blooded animals. Considering the delicacy of the estimations concerned and novelty of the methods used, I do not think Hill and I would have held tenaciously to the exact figure which we obtained, but as a matter of fact, the coefficient for sheep

hemoglobin has turned out to be approximately 4 as measured by Hartridge and Roughton (15), whilst as given above Seliskar and Maçela (20) make it 5 for human hemoglobin.

Hartridge and Roughton have shown by very beautiful methods that while the reaction

$$HbO_2 \rightarrow Hb + O_2$$

has a temperature coefficient of 3.8 (13), the reaction

$$Hb + O_2 \rightarrow HbO_2$$

has a coefficient of unity (14), being independent of temperature. Parenthetically let me say that the second part of their result appears to me to be of absorbing interest and may be explained by the assumption that every molecule of oxygen which impinges on a certain well defined region of the hemoglobin molecule becomes attached. If we are correct, then, that the hemoglobin which we investigated has a temperature coefficient approaching 4 for its reaction with oxygen, we must face the second alternative and ask ourselves—is the hemoglobin of say the frog different from that of the mammal? And if so, what is the difference?

We have already seen that as between frog and man there is a fundamental difference between the equilibrium constants of their hemoglobins at 12°C. under identical conditions, but it may well be that the temperature coefficients of these equilibrium constants also differ, and indeed that the disparity may be of biological interest. The temperature of the higher mammals (apart from hibernating animals) undergoes very slight variations, if such variations are to produce any effect of significance on the dissociation curve clearly the curve must be very sensitive. On the other hand the frog, in some countries, may change in temperature perhaps 30°C. as between the winter and the summer in which case hypersensitiveness of its dissociation curve might be entirely out of place. Clearly it is a biological necessity that the oxygen dissociation in forms which exhibit a high degree of poikilothermicity should be adapted to the general conditions of the animal and therefore from the biological standpoint the factors to which the temperature coefficient of K must conform are quite different from those in homoiothermic animals.

We have no knowledge of the differences of response to hydrogen ion concentration of different hemoglobins if indeed such differences exist. In the case of hemocyanin—the copper pigment—however, Redfield and Hurd (23) have discovered a very striking contrast between the

hemocyanins of Loligo and Limulus polyphemus. Carbonic acid tends to expel the oxygen from the blood of Loligo as it would from human blood, but contrariwise carbonic acid (i.e., presumably increased hydrogen ion concentration) tends to increase the affinity of the pigment of Limulus for oxygen. Redfield and Hurd put forward an interesting correlation between the functions of the pigments in these two forms of life and their reaction to carbonic acid. The "spatial limits" of this article forbid a digression, however interesting, into the field of pigments other than the immediate relations of hemoglobin.

HEMOGLOBIN AS A STORE OF OXYGEN. The fact that hemoglobin occurs in many places in the animal kingdom in which it is quite immobile and therefore cannot be used for purposes of transport, led to the idea that it was used as a store, so that in cases of necessity the tissue, frequently an important one, should have something on which to fall back. I do not know quite to what extent I must bear the responsibility of this conception, having been guilty of the following sentence (9): . . . . "it is suggested that in certain low forms of animal life in which hemoglobin exists in the substance of the nervous and muscular systems, it is retained as a 'store' from which the oxygen can be withdrawn in cases of extreme need which might result either from partial asphyxia or great functional activity: by a transition in which the hemoglobin is placed in the fluid round the organ, a functional respiratory circulation of hemoglobin might be evolved in forms in which the conditions for the oxygen to be discharged into the tissues are favourable. Such favourable conditions are rise of temperature and rise of CO_2 tension." In reviewing this sentence from a distance in the light of the knowledge gained during the fifteen years since it was written, one sees that it contains certain assumptions which demand some examination.

1. There is the underlying assumption not definitely stated, that hemoglobin is regarded as a store merely as an alternative—because hemoglobin is not a carrier in such and such cases, it may be a "store"—but as such the suggestion demands no more consideration than any other alternative which could be thought of. At the present time the most obvious would be a catalytic function.

2. The second assumption is that hemoglobin exists in stationary cells in lower forms of life than those in which it is to be found in circulation. The phase "certain low forms of life" is not a very easy one to dissect, and at the risk of being accused of cowardice, I refuse to attach any exact meaning to it. Certain it is that when I wrote it, the issues did

not present themselves which now occur to me as I sit down to criticise it. Is a larva a "low form of life" as compared with the imago? If it is I would mention two larvae in which hemoglobin occurs and in each of which the function of the pigment *qua* store may be canvassed. They are the larvae of Chironomus and Gastrophilus. It has been contended that the larva of Chironomus sinks to the bottom of lakes, where the water is devoid of oxygen and lives there for considerable periods of time in virtue of the oxygen in its alleged store of hemoglobin.

The view that hemoglobin in Chironomus and Planorbis is a store and not a carrier has been discussed at length in a paper from Krogh's laboratory by Miss Leitch (19). She finds that the animals neither store oxygen in the sense in which Miall meant it (he was thinking of his experiment where a larva lived for five days in boiled water) nor do they use it carefully.

Hemoglobin has also been regarded as a store in the worms, concerning which claim Miss Leitch gives the following quotation:

> A great number of worms are mud-dwellers. That their great power of resistance to lack of oxygen is correlated with their life in this badly oxygenated medium can scarcely be doubted. Bunge has expressed the suggestive idea that, only because of their having passed through the "preparatory school" of oxygen-want as mud-dwellers are worms capable of living quite anaerobically in the oxygen-free alimentary canals of higher animals. On the other hand, Lankester has tried to correlate the normal habitat of worms with the formation of respiratory pigments, and for example, expresses the view that only its wealth of hemoglobin enables the Annelid, Tubifex, to live at the bottom of the polluted water of the Thames. Probably, in fact, the two apparently contradictory aspects can be referred to one cause, namely, that the adaptation to a medium poor in oxygen led, on the one hand, to a high capacity for anaerobic life, and on the other, to oxygen-binding pigments, the possession of which must be of great advantage under just these circumstances.

In this connection there seems to be some evidence of hemoglobin acting as a store in the blood of Arenicola, this is based on the fact that the total oxygen capacity of that worm's blood is about equal to the whole quantity of oxygen that it would require during the hours of low water, when the worm is shut up in its hole (7). This conception demands a further study of the conditions of diffusion and the consequent oxygen supply in the moist sand. It does not preclude the use of hemoglobin as a respiratory pigment, and therefore it carries us into that most fascinating of all territories, the region in which an organ is changing its function, in which the organ is therefore working in a dual capac-

ity one phase of which is predominant at one time and another at the next.

HEMOGLOBIN AS A BUFFER. The extent to which hemoglobin can act as a buffer depends upon the quantity present. In blood it is of course present in great concentration consequently its buffering power is of a high order, in the tissues the concentration is low relatively to that of other proteins. The evidence goes to show that in muscle for instance the buffering is quite satisfactory apart from any question of hemoglobin. It must be remembered however that the circulating blood buffers the tissues and because hemoblobin buffers the blood, it indirectly performs the same function for the muscles and other organs.

TABLE 1

ANIMAL	POSITION OF α BAND IN ÅNGSTROM UNITS		SPAN (S) IN ÅNGSTROM UNITS S = A − B
	A, O_2Hb	B, COHb	
Man	5764	5710	54*
Arenicola (mean)	5746	5698	48
Lumbricus	5755	5720	35
Planorbis	5746	5708	38
Chironomus	5777	5727	50
Pigeon	5762	5710	52
Carp	5762	5716	46
Tortoise	5766	5717	49
Fowl	5769	5718	51
Lizard	5762	5715	47

* The above figures are all based on the assumption that the span for man is 54 A.U. As this figure is probably somewhat too low, they contain a systematic error and are subjected to multiplication by a factor slightly over 1, but as yet undetermined.

SPECIFICITY OF HEMOGLOBIN. The hemoglobins in various forms of life have been shown in the above discussion to differ in two properties, namely, their power of uniting with oxygen and the effect of temperature on that power. The reader must not carry away the impression that these are the only known differences or that they were the first to be discovered. They happen to have been treated first in this paper only because the argument fell out that way. That there is a difference between different forms of hemoglobin may be shown in a number of ways. One need only cite the numerous observations on the crystalline form (especially the monumental study of Reichert and Brown (24)),

on the solubility by Heidelberger and Landsteiner (18), on the gas combining properties by Haldane and his pupils (10), on the spectrum by Vlès (26) to assure the reader that hemoglobin is not uniform throughout the animal kingdom—or even throughout the vertebrata. The differences which exist are shown in table 1 in a particularly quantitative way.

Table 1 is a comparison of the relative position of maximum density of the oxy- and carboxy-hemoglobin bands in the spectra of human hemoglobin and those of numerous submammalian forms of life (2).

The variations in such phenomena as the crystalline form, the solubility, and the spectrum have not yet been fitted into any scheme which correlates them with the functional purpose which hemoglobin serves in the respiration of the organism. Yet such variations have a significance of their own. This significance has less to do with the iron containing part of the molecule than with the protein, for there are many things which are true of hemoglobin that may also be true of all the other proteins of the body; in the case of hemoglobin they may be discovered because the globin possesses a label the hem on which the story writes itself and may be read by all who have eyes to see. Once that story has been read completely methods may be devised for the discovery of its applicability to proteins generally and in the revelation of this story, no less than in the evolution of the respiratory function, may lie the significance of hemoglobin in the invertebrates.

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EDEMA AS A PROBLEM IN PHYSIOLOGICAL REGULATION

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Claude Bernard (1) in his *Leçons sur les Phénomenès de la Vie*, published in 1878, summed up his concept of physiological or organic regulation in the following words: "tous les mécanismes vitaux, quelque variés qu'ils soient, n'ont toujours qu'un but, celui de maintenir l'unité des conditions de la vie dans le milieu intérieur."

Nor was this a "vitalistic" conception of regulation since Claude Bernard was careful to point out that it opposed all of the contrary conceptions of the vitalists. It may be taken to imply that regulation is a conservative process, necessary for the free and independent life of the organism, developed in the course of evolution, and carried out as the resultant of a multiplicity of stimuli and of reactions which follow as necessary consequences of the stimuli.

Although the advance in our knowledge of the intimate workings of the local mechanisms in the body has been greater than it has been with respect to the process of physiological regulation, it is evident that the more general point of view of Claude Bernard has not been lost sight of. It has been followed by Krogh (2) in his studies of the capillaries, by Haldane (3), in his analysis of the respiratory process, and by Yandell Henderson (4) in his review of the physiological regulation of the acid-base balance of the blood.

Regulation of volume in the organism:[1] L. J. Henderson (5) pointed out that the regulation of volume in biology has remained without physico-chemical analysis, although "from the standpoint of physical science it is perhaps the most unusual and fundamental of all organic regulations." It is clear that the regulation of volume in the organism fulfils the conditions laid down by Claude Bernard. It is a process necessary for "preserving constant the conditions of life in the internal environment." Moreover, it is bound up with the regulation of other conditions of life, notably the osmotic pressure of the internal environ-

[1] See also Oehme, C.: Ueber den Wasserhaushalt. Klin. Wochenschr., 1923, ii, 1–5.

ment, although as Henderson points out, it is, in the last analysis, independent of the latter.

There is, as stated by Haldane (3) for physiological processes in general, a constant tendency on the part of physiological activity to disturb the volume relations in the internal environment, and the regulatory process acts to maintain a dynamic balance between the disturbing and restorative activities. It is a truism that equilibrium in the physico-chemical sense, occurs in the organism only with death. The condition of balance between varying, shifting, and opposing forces, which is characteristic of living processes in general, is variously spoken of as a "dynamic equilibrium," a "correlated state," or a "steady state."

That there is a steady or correlated state in volume relations in the organism is manifested in the fact that the total bodily volume and the internal distribution of bodily fluids both show an apparent stability, in spite of the continual interchange of fluids between "the infinite assemblage of phases which make up the organism" (5).

Edema as a disturbance in regulation of volume: The concept of edema as a disturbance in regulation of the volume of the fluids of the internal environment does not seem to have had the consideration it deserves. Recent reviews (6), (7) of edema have dealt with the subject mainly from the standpoint of the physico-chemical mechanisms involved, rather than from the standpoint of their regulation. So with most of the recent studies on edema. The monograph of Eppinger (8) is an exception, in that his thesis is to the effect that regulation is achieved through the medium of the internal secretion of the thyroid gland. His conception is of the greatest importance, even though the evidence so far presented does not seem to justify the belief that the regulatory mechanism has in fact been elucidated.

That edema is actually a manifestation of a disturbance in physiological regulation is a self-evident fact, if the concept of regulation be accepted. In spite of the apparent stability in bodily volume and in the volume relations within the internal environment, there are constant fluctuations in these conditions as the result of the temporary ascendency of the volume-disturbing or of the volume-restoring forces. It is only when there is a shift of volume to the tissues, tissue spaces, or bodily cavities, sufficient to become recognizable as a gross deviation from what we are pleased to term normal, that clinical edema exists. It may also be pointed out that an excessive shift of volume from the tissues and tissue spaces, and finally out of the organism, occurs in the condition known as anhydremia (9).

A quantitative conception of edema: It thus becomes apparent that edema is a quantitative rather than a qualitative deviation from the normal, and that there is no sharp line of distinction between the normal and the abnormal. Baird and J. B. S. Haldane (10) and Adolph (11) have shown that the behavior of fluid in the organism may be markedly influenced by the ingestion of salts, even to the point of gross edema in a normal subject. In such a case edema can be interpreted only as a response of a normal organism to unusual conditions. It is readily demonstrated, moreover, that bodily weight, and hence bodily volume, may be varied at will, within certain limits, by varying the amount of salt ingested. This fact is now commonly taken advantage of in the dietary control of obesity.

When edema occurs in the presence of pathological conditions, and in the absence of such factors as would provoke the same response in a normal individual, it may be considered as being due to a disturbance or perversion of the regulatory process itself, or to a limitation of its capacity because of alterations in the local conditions through which regulation is effected. The regulatory mechanism is able, within wide limits, to make up for defects in its component parts; but if its capacity is limited by conditions due to disease, its quantitative response to conditions such as the amount of salt ingested may be exaggerated. Thus an abnormal individual's capacity for maintaining normal volume relationships may be sharply reduced, so that daily ingestion of an amount of salt insufficient to provoke an unusual response in a normal individual may be sufficient to determine the occurrence of edema.

The reserve capacity of the regulatory mechanism, although rarely taken into consideration, probably accounts for much of the contradictory evidence based on purely physico-chemical data. It seems to explain the fact that edema may, and does disappear, without any demonstrable changes in the balance of forces which seem to have determined its appearance. The multiplicity of ways in which the regulatory mechanism may exert its effects, and the difficulty of defining the capacity factor, find expression in the elusiveness of the phenomenon known as edema. The existence of the capacity factor is implied in the use of the German term "oedembereitschaft," which may be defined as a reduced capacity of the regulatory mechanism, without demonstrable edema. If edema were a similar term designating the underlying condition rather than its manifestations, we might speak of "compensated edema" and "uncompensated edema." It is just as important to conceive of the organism as a whole as compensating for defects in its

volume-regulating mechanisms, as it is to conceive of it as compensating for defects in its circulatory, respiratory, or metabolic mechanisms.

The concept of edema as due to a perversion of the regulatory mechanism itself, rather than to a reduction in its capacity through defects in its component parts, deserves some consideration, although it does not appear probable that such a perversion is a frequent occurrence. The most suggestive example of the possibility of such a perversion is seen in cases of the so-called angioneurotic edema, where it may be that the nervous system is at fault.

The goal of physico-chemical studies: The goal of physico-chemical studies in edema is the analysis of the conditions and forces involved in determining the distribution of fluids in the body, to be followed by a synthesis of the results into a composite picture leading to a clear and complete understanding of the interaction of the factors concerned. The method of physico-chemical analysis in this problem is extraordinarily complex. Not only is the number of variable factors large, if the organism as a whole is considered, so large in fact as seemingly to defy mathematical analysis or human comprehension, but it is by no means certain that all of the variable factors of importance have been recognized. It is certain that the quantitative if not the qualitative importance of some of the factors is not yet understood. Many years of patient effort will undoubtedly be required to elucidate some of the fundamental physico-chemical laws which must be understood before the method of physico-chemical analysis can achieve success.

Thus it is becoming increasingly more evident that much of the problem of fluid behavior in the body depends upon an understanding of the physical chemistry of the proteins. The recent work of Loeb (12) while it has marked a brilliant advance, has served even more to call attention to the deficiencies still existing in our knowledge of this subject.

Notwithstanding the difficulties inherent in the physico-chemical method and in the problem of edema, it is of the greatest importance that analytical studies be pursued. It is a method by which systematic progress can be made toward an understanding of bodily mechanisms, and it may at any time make possible therapeutic interference with a disordered mechanism. The mechanisms are the agents through which physiological regulation becomes effective, and cultivation of their study may lead to a short cut to the solution of the control of the regulatory process.

Limitations of the physico-chemical method: On the other hand one

must be very guarded with respect to his hopes for the immediate, or even of the ultimate success of the method of physico-chemical analysis and synthesis, in so far as the reaching of the goal of complete understanding is concerned. It is by no means certain that elucidation of the significance of the local forces involved, from the purely physico-chemical standpoint, will lead to the solution of the problem of regulation, which is, after all, of primary importance. The method of analysis may not be capable of distinguishing cause from effect, and may even lead one astray. The organism as a whole is something more than the sum of its component parts.

The problem of diabetes, long studied from the viewpoint of the chemistry of intermediate carbohydrate metabolism, has recently had brilliant success, not from this standpoint, but from the standpoint of regulation. Just so may some understanding of the regulation of volume be reached, long before the intermediate steps in the process are determined. In both cases study of the intermediate steps is of great importance. Knowledge has however at times been advanced more rapidly as the result of work based on intuition, or on a new point of view, than by the application of the systematic method alone.

The exchange of fluids in the body: From the physico-chemical standpoint there has been much recent progress in our understanding of the local conditions which influence the exchange of fluids between the capillaries and the tissue spaces. The trend of modern thought, as supported by experimental evidence, is to the effect that such exchange takes place in accordance with well defined physico-chemical laws, and that there is no reason at present for postulating any specific secretory or absorptive powers on the part of the capillary endothelium.

It should therefore be possible, were the necessary data procurable, to consider any selected area in the body as a physico-chemical system, to determine the variables and the constants of the system, and to express these in terms of one another. We may now speak with some confidence only of the system which is made up of blood, capillary wall, and tissue fluids, and may predict the occurrences dependent on the physico-chemical characteristics of the system. We are still at a loss, even when this is done, as to the various ways in which this local system is affected by the more general regulatory processes of the organism.

Balance between osmotic and hydrostatic pressures: Starling (13) in 1896 first stated his concept of the forces concerned in the exchange of fluids between the capillaries and the tissue spaces, and this con-

cept is fully developed in his later writings (14). Previous to the work of Starling it was the belief, based on numerous determinations by the freezing-point method, that the tissue fluids were in osmotic equilibrium with the blood. The error was due to the fact that the osmotic pressure of the proteins in blood plasma, for example, has a magnitude of less than one per cent of the total osmotic pressure of the plasma, or well within the limit of error of the freezing-point method.

Starling's contribution was to the effect that the balance of forces in question depended not upon osmotic equilibrium, but upon osmotic dysequilibrium. He made the observation that the osmotic pressure of blood serum, when dialyzed against a protein free filtrate from the same serum, is from 30 to 40 mm. of mercury, and that this pressure corresponds very closely to the average hydrostatic pressure in the capillaries. His concept may be stated in his own words:

> So long as any difference in composition exists between the intra and extravascular fluids, so long will diffusion currents be set up, tending to equalize this difference . . . . we may conceive that there is normally a balance in the capillaries between the process of exudation and absorption, the former being conditioned by the capillary blood pressure and the latter by the difference in protein content, and therefore of osmotic pressure between the blood plasma and tissue lymph. A rise of capillary pressure will upset this balance in favor of transudation and the blood will become more concentrated, whereas a fall of pressure will turn the scale of absorption and the volume of blood will be increased at the expense of the tissue fluids (15).

The observations of Starling have been amplified and extended by Krogh (2) and more recently by Schade and Claussen (16), and their conclusions are entirely in accord with his. It would appear to the writer that the concept is sound, that the forces postulated by Starling are beyond doubt present and active in the manner described by him, and that a diminution in plasma proteins, or an increase in capillary pressure may in fact determine the occurrence of edema. The difficulties which have arisen with regard to complete acceptance of the concept are no doubt due to the as yet hidden means by which the organism is able to regulate volume, and to the fact that not all of the factors concerned in the balance were considered by him. These factors are not even now capable of direct and simultaneous determination. A further difficulty recognized by Starling resides in the impossibility of explaining the absorption of protein-rich exudates from the tissue spaces or from the serous cavities on the basis of his concept. The most obvious way of avoiding this difficulty is to assume that the proteins are either

broken up before being absorbed, or that they are absorbed only through the lymphatics. There are data which indicate both mechanisms but the point is far from being settled.

The Donnan equilibrium: F. G. Donnan (17) in 1911 formulated and verified experimentally the conditions which exist at equilibrium when two solutions are separated by a membrane permeable to some, but not all, of the ions present. His formulation, known as the Donnan equilibrium, expresses in a quantitative manner the influence of the non-diffusible ions on the distribution of the diffusible ions on the two sides of the membrane. Although Willard Gibbs (18) had previously derived, from thermodynamic considerations, the relationships involved, these principles did not find their way into physiological literature until after Donnan's publications.

It is evident that the Donnan equilibrium is of great general interest in physiology because such membranes as exist in the body are as a rule more or less impermeable to proteins, but permeable to water and to at least some of the inorganic ions present in the body cells and fluids. The Donnan equilibrium is here of interest from the standpoint of its bearing on the problem of fluid exchange in the body, and more particularly on account of the extension and clarification of the concept of Starling which it has made possible.

As has been seen, Starling called attention to the balance which exists in the body between hydrostatic pressure in the capillaries and the osmotic pressure of the plasma proteins, and he also recognized the importance of diffusion pressure as tending towards the restoration of a disturbed equilibrium. Donnan added a fourth source of energy, namely, that determined by the difference in electrical potential on the two sides of the membrane.

From the standpoint of physiology the most important result of the Donnan concept has been the correlation of the results of the action of these forces. At equilibrium there may be detected, by appropriate means, differences in diffusion potential, osmotic pressure, hydrostatic pressure, and electrical potential. Examination of any one of these factors alone would lead to erroneous conclusions, as in fact it has done in physiological studies. That it is now clear that the forces must be considered in their relation to each other is due to the concept of Starling and to the extension of his concept by the principles expressed in the Donnan formulation.

Thus the hydrogen ion, although freely diffusible, is seen to be under the control of forces other than diffusion pressure, and under physio-

logical conditions the tendency is toward differences in, rather than equality of, hydrogen ion concentration in cells and in body fluids of different protein concentration. Wherever the protein concentration is higher, there will the hydrogen ion concentration tend to be higher. Conversely, the concentration of the chloride ion tends to be lower wherever the protein concentration is higher. The demonstration of this principle has served to explain numerous observations as to the inequality of distribution of such ions in the body.

Although it has now been established (19) that the Donnan formulation is applicable to the study of at least certain relationships in the living organism, and while the tendencies indicated above have been derived from this formulation, a reservation should be made at this point. We have seen that equilibrium is not attainable under physiological conditions, although it is probably most nearly attained in vivo between the red blood cells and the plasma, and to a lesser extent between the plasma and the tissue fluids. On account of the continual disturbances of equilibrium in the body it is of importance that deductions from the Donnan formulation as to physiological conditions be made with great care.

Balance of forces concerned in the exchange of fluid: Of the four forces considered above, namely, diffusion pressure, hydrostatic pressure, osmotic pressure, and difference in electrical potential, it is impossible to separate out any as being the most important in determining the direction and rate of flow of fluid through body membranes. All of these forces are constantly being disturbed by physiological activity, and according to general laws their tendency in regaining a balance is to do so at the least expenditure of energy. Thus one may momentarily be of most, and at the next moment of least importance in the continually shifting balance. Again we see that the balance, and not the individual force, determines the conditions at any given moment.

There may be added a word as to electrical potentials, since this factor was not considered by Starling. The significance of this force was appreciated by Donnan, and has been studied experimentally by various investigators, more recently Mudd (20). There can be no doubt, either from theoretical considerations, or from experimental results, that differences in electrical potential may influence the direction and rate of flow of fluids through such membranes as exist in the body. It also seems clear that differences in electrical potential are set up as the result of physiological activity, and that these differences play their part in the balance of forces. It is impossible, however, at the

present moment, to determine the physiological significance of this force as apart from the other forces concerned.

Edema and the Donnan equilibrium: We have seen that the Donnan formulation has clarified the conception of the balance of physico-chemical forces concerned in maintaining a steady state in fluid exchange between the capillaries and the tissue spaces. We have further seen from our quantitative conception of edema, that the occurrence of edema is in no sense an indication of any fundamental change in the manner in which the steady state is maintained, but indicates rather a steady state of a system with a new configuration. There remains to be reviewed the light thrown on the problem of edema by the principles embodied in the Donnan formulation.

It has been shown by Loeb, Atchley and Palmer (21) that in edema the conditions on the two sides of the capillary wall represent those of a simple membrane equilibrium. From the data of these investigators Van Slyke, Wu and McLean (19) were able to show that the relationship of the composition of edema fluid to that of the blood plasma approximates that postulated from the Donnan formulation. Study of the aqueous humor of the eye, and of the cerebro-spinal fluid (22) has indicated the same relationship. Presumably the same condition holds as to the equilibrium between blood plasma and normal tissue fluids, although the latter have not been obtainable for analysis.

The important point at this juncture is that in none of these instances does volume enter into the calculations, and that volume conditions, at equilibrium, are independent of the Donnan formulation. In fact, Willard Gibbs (18) showed that when equilibrium is established in a system, capillary and gravitational factors being absent, the volume of the phases is irrelevant to the equilibrium conditions. But volume is important as one of the conditions involved in an analysis of the process of reaching equilibrium, and in the presence of further limiting conditions the Donnan formulation may be used as a basis of reasoning with respect to volume changes. In its present state, however, the chief service of the Donnan equilibrium in the problem of edema has been the clarification of thought with reference to membrane equilibria in the body in general, and the explanation of the results obtained from simultaneous analyses of edema fluid and blood plasma. It is evident that the Donnan equilibrium does not, and cannot explain edema.

The state of the capillary membrane: Although the conditions on the two sides of the capillary wall have been shown to be those of a simple membrane equilibrium, such as exists on the two sides of a collodion

membrane, the possibility of alterations in the membrane itself must not be lost sight of. In fact alterations in capillary permeability have for a long time held a major place in various theories of edema (23).

The term capillary permeability has been somewhat loosely used, and it is necessary to define it more accurately. The capillaries are apparently freely permeable at all times to water, to most if not all monovalent ions, to such substances as glucose or urea, and to most gases in solution, such as oxygen. They are apparently somewhat less permeable to certain bivalent ions, such as sulphates. There is no evidence of variations in the permeability of the capillary walls to such substances as have been mentioned, and it can readily be shown from simple thermodynamic considerations that alterations in permeability, in the quantitative sense, affecting only the rate at which substances pass through the capillary walls, will not affect direction of transfer of fluid, and hence will not have any appreciable effect on volume conditions.

The term capillary permeability has been used by Starling and Krogh in a more restricted, and in a physiologically more significant sense. To them it designates the extent to which the capillary walls allow proteins to pass through. A change in the capillary permeability to proteins, which results in a transfer of proteins to the tissue spaces, will lead to a diminution of the difference in osmotic pressure between plasma and tissue fluids, and hence may lead to edema. Such a condition in fact occurs in inflammatory edema and in the edema due to paraphenylenediamine poisoning, in which the edematous fluid has a composition approaching that of normal plasma (24). On the other hand it is possible to conceive of a diminished permeability to proteins, resulting in a conservation of osmotic pressure difference, and hence tending to hold the fluids in the capillaries.

Asher's recent work (25) seems to offer direct evidence that the nervous control of the capillary wall may have an effect on its permeability, and Petersen and his collaborators have made use of this concept in their studies in endothelial permeability (26). The latter believe that there may be variations in the filtration of colloids through the capillary wall according to the state of activity of the endothelial cells, and they state that "filtration must be considered as an expression of a cellular function in which the state of activity or rest of the cells plays a vital rôle." They bring forth evidence to show that the permeability of the capillaries to proteins may be markedly influenced by epinephrin. This indicates a nervous control under the influence of the sympathetic system.

The observations of Morawitz and Denecke (27) are of interest in connection with capillary permeability, although the interpretation of the phenomenon involved is by no means clear. They determined the total solid content of serum from blood obtained from an arm vein after constriction of both the arterial and venous circulation in the arm for a period of ten minutes, and compared the findings with those obtained before constriction. While in normal individuals the blood thus shut off in the arm becomes diluted to an extent of from four to six per cent, it becomes more concentrated in certain conditions associated with edema, and in conditions such as scarlet fever, in which they assumed the capillaries to be injured. It will be seen that since this phenomenon involves a change in the direction of flow of fluid, it cannot be explained on the basis of the conception of capillary permeability outlined above, although Morawitz and Denecke regard it as a test of capillary function.

The flow of lymph: It is clear that at least under certain circumstances the flow of lymph from the tissues plays a part in the regulation of volume of the tissue fluids. Apparently this mechanism is not of importance in the resting condition, in at least certain tissues, for, as Asher (28) showed, the rate of lymph production and flow depends on tissue activity, and lymph flow from the extremities practically ceases when these parts are at rest.

Since the mode of entrance of fluid into the lymphatics is not clear it is impossible at present to analyze the mechanism of lymph production. Starling (14) has shown however that the protein content of lymph differs in different parts of the body, being lowest in the extremities. It appears therefore that proteins which are able to find their way out of the capillaries may also find their way into the lymphatics. As has been seen above, this is of importance in any consideration of fluid exchange between the capillaries and the tissue spaces, since the conceptions discussed do not provide for the transfer of protein through a membrane from regions of lower to regions of higher protein concentration, and hence do not include reabsorption of protein-rich exudates by the capillaries.

In man, localized edema frequently follows injury or blocking of the lymph channels. The rôle of alterations in the lymph flow in the production of generalized edema, however, is not known. Cohnheim and also Klemensiewicz, believed that any increase in the transudation of fluid from the capillaries was followed by an increase in the lymph flow, but that edema occurs in spite of this, because of the limited

capacity of the lymphatic system. The possibility of such a determining factor for the occurrence of edema has recently been restated by Rusznyák (29).

The function of the lymphatics, apart from their absorptive functions, may be conceived of as having partly to do with the regulation of volume of the tissue fluids, and partly with the removal of protein from the tissue spaces, the latter of course influencing also the former.

The rôle of the cells: There remains to be considered the rôle of the tissue cells, both as to their direct part in the process of edema and as to their influence on the regulation of volume.

Within certain limits the cells themselves are capable of volume changes, and they are undoubtedly subject to continued slight changes in volume, due to variations within the cells and in their environment. Under pathological conditions there are wider fluctuations, as seen particularly in anhydremia (9), in which state a large proportion of the bodily weight may be lost. Krogh (2) attributes to the cells the source of at least a part of the fluid that passes into the blood vessels following hemorrhage, since the normal volume of tissue fluids does not seem to be large enough to account for the increased volume of the circulating blood. Whether significant changes occur in the opposite direction, that is, in increase of cell volume, and whether such changes are of importance in general edema, is still debated. Fischer (30) believes that this is the primary change and that the accumulation of fluids outside the cells is a secondary phenomenon.

It seems probable on general grounds that the various stimuli leading to changes in volume conditions in the internal environment originate from the cells, and that such changes occur as a response to the needs of the cells. The results of such a response are seen in the increase of lymph production accompanying tissue activity referred to above. The probability is that such stimuli are chemical in nature.

Regulation of concentration of specific ions and of osmotic pressure: We have already mentioned above that the regulation of volume is bound up with the control of the osmotic pressure of the internal environment. The whole matter of regulation of volume might be approached directly from this standpoint, but we shall here merely call attention to the fact that the organism does regulate the osmotic pressure of its internal environment, thus preserving constant this condition of life.

One stumbling block appears at present to be insuperable from the purely physico-chemical standpoint in a consideration of edema, namely,

the inability to explain, on physico-chemical grounds, the differences in the behavior of the organism toward sodium and potassium. In simple physico-chemical systems these two substances behave very much alike, but their distribution in the body, and the reaction of the organism to their ingestion show no such similarities. There is a great excess of potassium over sodium in all of the body cells, and in most food materials. The conditions in the body fluids are reversed, the concentration of sodium in the plasma, for example, being about seventeen times that of potassium (31). The organism, normal or pathological, responds to increased ingestion of sodium salts by increase in volume, this reaction being more marked in individuals with a tendency to edema. No such response to potassium salts is seen (11) even in patients with edema (32).

Since the classical work of Widal (33) much has been made of the influence of chlorides on the behavior of fluid in the body, particularly in edema. The data upon which the conclusions are based are derived mainly from the study of the effects of the ingestion of sodium chloride. It now appears that the results of giving potassium chloride are quite different (32), and it is likely that the prevalent opinions concerning the rôle of chlorides in edema would not have been so firmly established, had the quantitative determination of sodium and potassium been as simply carried out as is that of the chlorides.

The recent work of Collip (34) has given ground for belief that the regulatory mechanism for the concentration of calcium in the internal environment is about to be elucidated. A rational explanation of the behavior of sodium and of potassium is much to be desired, and the difference can only be attributed for the present to physiological regulation, the mode of regulation being left as an unsolved problem.

Volume of the blood: The maintenance of a fairly constant volume of the circulating blood appears to be of major importance in "preserving constant the conditions of life in the internal environment." In spite of an infinite number of possibilities for the blood to become diminished or increased in volume to a point incompatible with life, such an event is uncommon, except as the result of accident.

The forces which tend to maintain the blood at a relatively constant volume are often thought of as being inherent in the blood itself. Thus the question is debated as to whether the plasma proteins achieve this end by virtue of their osmotic pressure or of their power to bind water, by hydration (35), (36). It is obvious that neither of these conceptions can suffice to explain even the meeting of the varied

conditions of every day life. And when there is a sharp drop in plasma proteins, as in the case of hemorrhage, both conceptions break down completely, for the volume tends to the normal in spite of the very great change in the physico-chemical characteristics of the blood.

The occurrence of edema may indeed be conceived of as merely an expression of regulation of the volume of the blood. Following this conception, an essential for the life of the organism is a fairly constant volume of circulating blood, a secondary consideration being the volume of fluid in the tissue spaces and in the body cavities. If for any reason the organism is unable to dispose of excess fluid through the usual channels, there remain two possibilities; either the fluid remains in the blood vessels, a condition incompatible with life, or it passes into the tissue spaces, thus conserving the life of the organism. Further evidence that the volume of fluid in the tissue spaces is a secondary consideration is seen in the movement of fluid from these spaces and probably also from the cells into the blood stream following hemorrhage, a response which very frequently results in conservation of life. Moreover, as is well known, ingestion of a large amount of fluid under normal conditions causes no appreciable increase in the volume of circulating blood, but apparently does cause an increase in the volume of the tissue fluids.

Manner of regulation of volume: We have seen that the regulation of volume in the internal environment is bound up in a very complex way with the local physico-chemical characteristics of the organism, and with the regulation of other conditions of life. The necessity for correlation of these varied forces and conditions is strikingly apparent, for the existence of the organism depends upon such correlation.

The sources of fluid, and the ways in which the body disposes of it, were set forth by Claude Bernard (1). A recent review (37) shows that little has been added since then to clarify our understanding of the control exerted over this balance by the organism as a whole.

We may not therefore go much further than Claude Bernard did, in an attempt to visualize the manifold workings of the general process of regulation. He saw clearly that the nervous system bears the brunt of adjusting water intake and water loss in order to maintain a balance. The influence of nervous control on blood pressure, blood distribution, and renal activity has been too well demonstrated to allow doubt as to the importance of the nervous system as a correlating agent in all phases of volume control. The nature of the effective stimulus, of the center or centers through which reflex action occurs,

and of the method by which nervous impulses become effective in volume regulation must be left to the future to decide. The capillary bed, the intracapillary pressure, and probably the permeability of the capillary walls are subject to nervous control, and it may be through these controls that local volume conditions are regulated, the total volume at the same time being subject to the nervous control of renal activity.

Evidence is accumulating to the effect that there is a center in the hypothalamus which exerts a marked influence on the control of water balance in the body. Veil (38) has recently given extended consideration to this subject, and the experiments of Curtis (39) indicate that such a center in the hypothalamus may operate independently of the pituitary body.

There may be suggested the additional probability of chemical regulation as an important factor. Blood volume is markedly influenced by the oxygen tension of the atmosphere. Carbon dioxide and lactic acid production seem to influence the distribution of blood in the organism, and these factors would seem to have marked bearing on the regulation of fluid exchange in the tissues. Tissue activity, either through its mechanical or chemical influence has been found by Scott (40) and his collaborators to influence the interchange of fluid between the blood and tissue spaces.

For the possibility of coördination between nervous and chemical regulation reference may be made to the control of the capillary bed. Hooker (41) suggests in this instance that nervous control tends to restrict the capillary beds over the body as a whole, thus maintaining a tone to be played upon by chemical factors. A similar balance may play a part in the regulation of the volume of the internal environment.

Finally there is the possibility of a specific regulatory mechanism, depending upon an internal secretion, or a balance between two or more such secretions. This hypothesis, advanced by Eppinger (8) has much to commend it, in the influence that thyroid extract and pituitrin are known to have on the behavior of fluid in the body. The possibilities in this direction at present are however interesting only as a basis for speculation.

Edema as a symptom: Edema has been referred to in the above pages as a disturbance in volume relationship. It may now be considered as a symptom of disease, in its relation to the more common conditions with which it is associated.

The edema of heart failure: One of the most common causes of edema

is embarrassment of the peripheral circulation, due to heart failure. In this condition the edema is more marked in the more dependent portions of the body, as are other evidences of circulatory failure.

The concentration of the plasma proteins is as a rule not reduced in this condition, and may be increased. Correspondingly the osmotic pressure of the plasma proteins is within normal limits. Increase in capillary pressure to an extent sufficient to overbalance the osmotic pressure of the proteins would account for the edema, and indeed this now seems to be the most likely explanation. Whether an additional factor is needed, such as increased capillary permeability to proteins, due to asphyxia, as is assumed by Starling, is not yet clear. The concentration of proteins in subcutaneous effusions in heart failure is low (42), (43), a condition which certainly does not indicate increased permeability of the capillaries to proteins, but rather suggests a compensatory decrease.

The conclusion may be drawn that in heart failure the circulatory changes tend to increased volume of the tissues, but that the regulatory mechanism is generally sufficient to prevent gross edema in all except the more dependent portions of the body, where the influence of gravity becomes the determining factor.

A consideration of the way in which physiological regulation overcomes the ever-present influence of gravity may be illuminating at this point. From the viewpoint of the organism as a whole as a purely physico-chemical system it is difficult to explain why the body fluids do not at all times tend to settle to the more dependent portions of the body, as in fact they do settle after death. From this point of view, for example, the venous pressure in the foot should equal the venous pressure at the heart level, plus the height of a column of blood from the foot to the heart level, plus the added pressure necessary to overcome resistance to venous return. Such a venous pressure is seen in fact, in paralysis of the extremities, or as the result of conscious relaxation in the normal individual with the extremity in a hanging position (44); in both cases edema of the foot occurs (2). That it does not occur under normal conditions is due to physiological regulation of venous pressure in the extremities, this pressure being much lower than is to be expected from the foregoing considerations (45). Regulation, in this instance, is effected through the coöperation of the voluntary muscles of the legs together with the pump-like action of the joints upon the neighboring veins (44).

The edema of nephrosis: There is a condition formerly included under

the term of "chronic parenchymatous nephritis," but now often regarded as a separate clinical entity. On account of the striking degenerative changes found in the epithelial cells of the tubules of the kidneys at autopsy, Friederich Müller (46) in 1905 proposed the name "nephrosis" for this entity.

One of the outstanding features of this condition is a diminished concentration of the plasma proteins, first noted by Richard Bright (47). Since the distinction has been made between nephrosis and true nephritis the plasma proteins in cases of nephrosis have been studied by Epstein (48) and others (49). Whereas the normal concentration of the total plasma proteins is from 5.5 to 7.5 per cent, it is characteristically diminished in chronic nephrosis to 4 per cent or even less. Linder, Lundsgaard, Van Slyke and Stillman (50) have shown that this change is due to a decrease in the total amount of plasma protein in the body, and not to an "hydraemic plethora."

Coincident with this change in total protein concentration there is a marked change in the relationship of the individual protein fractions of the plasma. In the normal individual the serum albumin fraction is higher than the total globulin fraction, including serum globulins and fibrinogen, so that the albumin: globulin ratio is uniformly over 1 and usually in the neighborhood of 1.7 ± 0.3. In cases of nephrosis, due to an absolute decrease of the serum albumin fraction this ratio is sharply diminished, ranging down to 0.26 (49). The decrease in total protein content is almost wholly due to the decrease in the serum albumin fraction, the serum globulin remaining fairly constant and the fibrinogen being increased several fold (51).

As is to be expected, the decrease in protein concentration results in a decrease of the osmotic pressure of the plasma proteins. Schade and Claussen (16) have studied this feature, and find a decrease in this pressure, which they call "oncotic pressure." While they find the normal oncotic pressure of the plasma to vary from 21.4 to 27.6 mm. of mercury with an average value of about 25 mm., the values in patients with renal edema are under 20 mm., and may be as low as 11 mm. In nephritic patients without edema they found normal values.

Epstein (48), on the basis of his analyses of the plasma, and Schade and Claussen, on the basis of their osmotic pressure determinations, both reach the conclusion, that edema in nephrosis is due to a diminution in the osmotic pressure of the plasma proteins. It is clear that such a reduction in osmotic pressure should disturb the balance of

forces in a manner tending towards increased transudation, and the explanation therefore appears to be rational. It seems, however, that the organism through its volume-regulating function may be able to compensate for this defect, since there may be marked changes in the amount of edema without any demonstrable change in the osmotic pressure of the proteins.

The significance of capillary permeability in nephrosis is undetermined. The extremely low protein content of edema fluids in nephrosis, lower indeed than that of normal lymph from the extremities, suggests that the capillary permeability for proteins may actually be diminished in this condition, such a change being a compensatory process conserving the osmotic pressure difference.

The concept of increased permeability of the capillaries in nephrosis has been used by Marriott and Clausen (52), (53), (54) who have found a lowered surface tension of the plasma, the degree of lowering accurately reflecting the clinical progress of the cases. They have isolated a surface active substance from the plasma and from the urine, found only in true cases of nephrosis, and have found that when collodion membranes are treated with this substance in water, or with urine or plasma from cases of nephrosis, they become permeable to proteins. The exact significance of their work in the absence of their final publications is not yet subject to analysis. The low protein content of the edema fluid in nephrosis is difficult to reconcile with the idea of increased permeability due to a surface-active substance.

The edema of nephritis: Edema is a fairly constant symptom of acute glomerular nephritis. Its early appearance in the soft tissues about the orbit, rather than in the more dependent portions of the body, suggests that the factors underlying its appearance are general, and that its localization depends on the resistance of the tissues to an increase in the volume of the tissue fluids. Its frequent, but less constant, occurrence with the same distribution in the more chronic forms of nephritis leads to the belief that the nature of the edema is the same in both instances.

In no condition is the nature of edema more obscure. Accordingly the causation is a favorite field for speculation, the primary factors being attributed in turn to renal insufficiency, to increased capillary permeability and to changes in the tissue cells. Beckmann (43) has found that the protein content of subcutaneous effusions in glomerular nephritis is higher than in any other form of edema. While such fluids in nephrosis have as a rule a protein content of less than 0.1

per cent, those in glomerular nephritis are usually above 1.0 per cent. This difference suggests a difference in capillary permeability for proteins, but in the absence of normal figures it is impossible to determine in which case the permeability has been altered.

The thesis of Fischer (30) may be stated in his words. "The changes that characterize nephritis are colloid-chemical in nature and due to a common cause—the abnormal production or accumulation of acid and of substances which in their action upon colloids behave like acid in the cells of the kidney." A consideration of the evidence for and against this statement would be beyond the limits of this review. The importance of the colloids with respect to the fluid balance in the body has been emphasized above. The writings of Fischer have undoubtedly served to draw attention to the rôle of the colloids, but the weight of evidence today is certainly against his main contentions.

It should be observed that in many cases there is no sharp line of separation between nephrosis and nephritis. In the mixed cases, known under various names, the findings are irregular, and the clinical picture partakes of the features of both conditions. Analyses of edema fluids from such cases are reported by Beckmann (43).

Soda edema: Edema from ingestion of the salts of sodium, particularly sodium bicarbonate, is not uncommon, especially in the presence of various debilitating influences. It has been noted most frequently during alkali therapy of diabetic acidosis, and a recent case has been described as occurring during convalescence from pneumonia (55). From our previous discussion it would appear that this form of edema is simply an exaggeration of the normal response to ingestion of sodium salts, due to some limitation of the capacity of the regulatory mechanism resulting from disease.

Nutritional edema: Although not a new condition, attention was attracted to this form of edema by the fact that large numbers of cases were seen during the war in countries where there were enforced dietary restrictions. It has been variously known as "war-edema," "oedem-krankheit," and "famine edema." It appears to be due to dietary deficiencies, and more particularly to a diminished protein intake over long periods of time. The nature of the disturbance in volume regulation which it represents is not known.

Inflammatory edema: Localized edema is a common occurrence about areas of inflammation. This form of edema is attributed by Schade and Claussen (16) to increased permeability of the capillaries, since the exudate is rich in proteins.

Toxic edema: Edema, local or generalized, may occur as the result of poisoning or intoxication. A very interesting form of edema, due to paraphenylenediamine, has been studied by Tainter and Hanzlik (24), who find that there is an increase in capillary permeability resulting in the escape of whole plasma, including fibrinogen, into the tissues of certain regions. They also find that the edema may be prevented in experimental animals by any measures which reduce the systemic blood pressure below 85 mm. of mercury.

Angio-neurotic edema: This form of edema is of particular interest in that it illustrates the possibility of a perversion of the nervous control of volume regulation.

The problem of edema: The problem of edema has been attacked from many standpoints, some of which have been considered above. The writer has attempted to make clear his belief that the fundamental problem is that of the physiological regulation of the volume of the internal environment, including the blood and the tissue fluids. It appears that edema is not an entity in itself, due always to the same underlying causes, but rather that it is a symptom just as dyspnea, which may result from various disturbances of the regulatory process.

Different methods of approach to the problem appeal to different investigators. The physico-chemical method, which has been so fruitful in the past, may be expected gradually to disclose as yet hidden mechanisms. The more general approach from the standpoint of the organism as a whole, with an attempt to solve the nature of the regulatory process, to discover the unknown "influences" and the manner of their control, offers a tempting field for investigators. Meanwhile the more and more detailed observation of patients with edema, the search for and correlation of deviations from the normal may bring to light new phenomena, each being the starting point for further investigation. By a combination of the three methods, in all of their various ramifications, may one hope for solution of the problem and control of the condition of edema.

As an example of new phenomena whose elucidation by the experimental method may bring us nearer to an understanding of the nature of edema, there may be mentioned three extremely interesting observations. One is the discovery, by Clausen (53), of lowered surface tension of the blood serum in nephrosis with edema. Another is the observation of McClure and Aldrich (56) that salt solution, injected intracutaneously in patients suffering with edema, or with toxemia due to acute infectious disease, is absorbed much more rapidly than in normal

individuals. The third is the observation of Morawitz and Denecke (27) as to the movement of fluid in an extremity isolated from the general circulation. While we may not be certain that the explanations so far advanced for these three phenomena are correct, yet the pursuit of the nature of these phenomena and the discovery of new phenomena, are bound to shed much light upon the problem.

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